1-2011

Biodegradation Kinetics for 1,2-Dichloroethane and Ethylene Dibromide in Anaerobic Enrichment Cultures Grown on Each Compound

Rong Yu
Clemson University, yurong84@gmail.com

Biodegradation Kinetics for 1,2-Dichloroethane and Ethylene Dibromide in Anaerobic Enrichment Cultures Grown on Each Compound

---

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Environmental Engineering and Science

---

by
Rong Yu
January 2011

Approved by
Dr. David L. Freedman, Committee Chair
Dr. Cindy M. Lee
Dr. Ronald W. Falta

ABSTRACT

1,2-Dichloroethane (DCA) and ethylene dibromide (EDB) are among the top 15 chlorinated aliphatic compounds on the Agency for Toxic Substances and Disease Registry's Priority List of Hazardous Substances. Co-contamination of groundwater with EDB and 1,2-DCA resulted mainly from environmental releases of leaded gasoline; these compounds were added as lead scavengers. A microcosm study by Henderson et al. (*1*) with soil and groundwater from a site contaminated with leaded gasoline demonstrated anaerobic biodegradation of EDB at a higher rate, and to a greater extent, than 1,2-DCA. Both compounds were transformed mainly by dihaloelimination to ethene. The objectives of this study were to measure maximum specific growth rates, half saturation coefficients, and lag times in enrichment cultures that use 1,2-DCA and EDB as terminal electron acceptors; and to evaluate if the presence of EDB has an effect on the kinetics of 1,2-DCA dehalogenation, and vice versa. The effect of each compound on biodegradation rates of the other was evaluated at the high concentrations that may be found at industrial sites (e.g., >10 mg/L) and at the lower concentrations that have been reported at leaded gasoline sites.

Two enrichment cultures were developed; one was grown with EDB as its terminal electron acceptor, the other with 1,2-DCA. The cultures dehalogenated weekly additions of approximately 24 mg/L of EDB and 1,2-DCA. Lactate was provided as the electron donor and ethene was the predominant product from both compounds. The enrichment cultures were used as a source of inoculum in experiments designed to measure the maximum specific growth rate ($\hat{\mu}$) and half saturation coefficient (K_S) under different conditions: with EDB and 1,2-DCA alone, using their respective enrichment cultures as inoculum; with EDB alone, using the 1,2-DCA enrichment culture as inoculum; with 1,2-DCA alone, using the EDB enrichment culture as

inoculum; and with both compounds present together, in one case with the EDB enrichment culture as inoculum and another with the 1,2-DCA enrichment culture as inoculum. Both enrichment cultures grew on either compound, even though the EDB enrichment had never previously been exposed to 1,2-DCA and vice versa.

Based on batch depletion experiments performed at high concentrations of 1,2-DCA and EDB, the maximum specific growth rate ($\hat{\mu}$) ranged from 0.19 to 0.58$d^{-1}$for 1,2-DCA and from 0.30 to 0.45 d^{-1} for EDB, with somewhat lower rates for EDB when the 1,2-DCA culture was used as the inoculum source. Maximum transformation rates were 130 μM/d for 1,2-DCA and 74 μM/d for EDB. The half saturation coefficient for 1,2-DCA (5.7-15.7 mg/L, or 58-158 μM) was notably higher than what has been reported for polychlorinated ethenes (e.g., 1.6-3.9 μM for tetrachloroethene, 1.8-2.8 μM for trichloroethene, 1.8-1.9 μM for *cis*-dichloroethene) but similar in magnitude to vinyl chloride (63-602 μM); the higher K_S values occurred when EDB was present along with 1,2-DCA. The K_S values for EDB were considerably lower than for 1,2-DCA, with three of the four treatments at or below 15 μg/L (0.082 μM). Nevertheless, the K_S for EDB is two orders of magnitude higher than its maximum contaminant level (MCL; 0.00027 μM). In nearly all of the bottles used to measure $\hat{\mu}$ andK_S, the rate of consumption slowed down faster than what was predicted by Monod kinetics. At this transition point, EDB and 1,2-DCA levels reached a plateau or decreased at a considerably slower rate. To account for this behavior, the Monod model was modified to include a transition concentration (S_t), which was subtracted from the substrate (S) concentration. Without S_t in the model, the error associated with $K_S$was significantly higher for 1,2-DCA and EDB; the $K_S$value for 1,2-DCA did not change substantially or increased somewhat; and the $K_S$value for EDB was either unchanged or

decreased significantly. S_t levels ranged from 12.9 to 127.5 µg/L for 1,2-DCA and 0.5 to 9.2 µg/L for EDB.

Most importantly, in treatments when EDB and 1,2-DCA were both added, the EDB was always consumed first and adversely impacted the kinetics of 1,2-DCA utilization. In separate experiments with 1,2-DCA provided alone, dechlorination of 1,2-DCA was interrupted by adding EDB at a concentration more than 100 times lower than the remaining 1,2-DCA; use of 1,2-DCA did not resume until the EDB decreased close to its MCL.

Lag times prior to the onset of 1,2-DCA dechlorination ranged from 10-75 days, versus only 2-15 days for EDB. The longest lag periods for 1,2-DCA occurred in treatments that were inoculated with the EDB enrichment culture. Also, the presence of EDB with 1,2-DCA significantly increased the lag times prior to the onset of 1,2-DCA utilization. However, the lag time for EDB was not impacted by the presence of 1,2-DCA (regardless of the inoculum source).

Batch experiments were also performed at lower 1,2-DCA and EDB concentrations, similar to those found near the source zone of leaded gasoline spills. When the 1,2-DCA enrichment culture was provided as the inoculum, EDB was consumed first, reaching its MCL level in 11-22 days. In the treatment with only 1,2-DCA added, its MCL was reached in 28-45 days. With EDB present, biodegradation of 1,2-DCA started shortly after the EDB reached its MCL and the 1,2-DCA was consumed at an equivalent or slightly faster rate than in the treatment with 1,2-DCA alone, suggesting that prior exposure to EDB had a slightly beneficial impact. With the EDB enrichment culture as inoculum, EDB was consumed first, in the presence or absence of 1,2-DCA. The treatment with EDB alone was faster and reached the MCL first; however, the time required to reach the MCL was notably longer (34-38 days) than with the 1,2-DCA enrichment culture. The lag time prior to the onset of 1,2-DCA

biodegradation was 49-61 days, considerably longer than for the 1,2-DCA enrichment culture. In the treatment with both compounds present, biodegradation of 1,2-DCA started around the time when EDB reached its MCL. Overall, these experiments confirmed the preferential consumption of EDB over 1,2-DCA and that both contaminants can reach their respective MCL levels, regardless of the type of enrichment culture used as inoculum.

Evidence continues to accumulate for the need to monitor 1,2-DCA and EDB contamination of groundwater, especially at former leaded-gasoline site. Corresponding interest in remediation approaches is likely to increase. Bioaugmentation is a candidate approach for sites where monitored natural attenuation is infeasible. Although considerable information is available on cultures that can dechlorinate 1,2-DCA, most have not been tested for their ability to debrominate EDB. Of the two enrichment cultures evaluated in this study, the 1,2-DCA culture has the advantage of more rapid transition to 1,2-DCA after EDB is consumed. Additional information is needed on the ability of enrichment cultures to dehalogenate 1,2-DCA and EDB in the presence of persistent fuel hydrocarbons.

ACKNOWLEDGEMENTS

The assistance of Micah Freedman and Ashley Eaddy in developing and maintaining the enrichment cultures is gratefully acknowledged. Bridget O'Donahue and Dr. James Henderson performed preliminary experiments that were needed to design the final experiments. Partial funding for this project was received via a DuPont Young Scientist Award to Dr. Yanru Yang, who assisted with the design of the experiments. Dr. Matthew F. Verce provided helpful guidance on use of AQUASIM and the best approach to modeling.

I would like to thank Dr. David Freedman for his guidance over the past three years and countless hours of help with my research. I am very fortunate to study under Dr. Freedman because of his uncanny ability to relate science to everyday life making him an excellent teacher and mentor. I would also like to thank the members of my committee, Dr. Cindy Lee and Dr. Ronald Falta for their participation during this process. I would like to also thank my fellow lab mates:Huifeng Shan, Vijai, Han Wang, Christina Anderson,Mike Hickey, Francisco Barajas, and Anthony Reid for their help and company while conducting experiments.

I must thank my parents for their endless help and encouragement. Last, but not least, I would like to thank my boy friend Tianhongfor his unending support.

TABLE OF CONTENTS

Page

TITLE PAGE i

ABSTRACT ii

ACKNOWLEDGEMENTS vi

LIST OF TABLES ix

LIST OF FIGURES x

ABBREVIATIONS xvi

1.0 INTRODUCTION 1

1.1 Overview 1

1.2ResearchObjectives 3

2.0 MATERIALS AND METHODS 4

2.1 Chemicals and Media 4

2.2Analytical Methods 5

2.3 Enrichment Cultures 6

2.4Batch Kinetics at High Concentrations 6

2.5Biodegradation of 1,2-DCA and EDB at Concentrations Representative of Leaded Gasoline Spills 11

3.0 RESULTS 11

3.1 High Concentrations of 1,2-DCA and EDB 11

3.2Effect of Low EDB Levels on 1,2-DCA 15

3.3Biodegradation of 1,2-DCA and EDB at Concentrations Representative of Leaded Gasoline Spills 16

4.0 DISCUSSION 17

5.0 CONCLUSIONS 21

TABLE 23

FIGURES 24

APPENDICES 29

Appendix-1:Pathway for 1,2-DCA and EDB Biodegradation Under Anaerobic Conditions 30

Appendix-2:Enrichment Cultures 31

Appendix-3:Magnitude of Electron Donor Additions 36

Appendix-4:Evaluation of Strategies for Adding 1,2-DCA and EDB 40

Appendix-5:Percent Recoveries of Ethene, VC, VB and Methane 44

Appendix-6:Comprehensive Results for theHigh Concentration Testswith1,2-DCA and EDB 46

Appendix-7:Mineral Medium Controls and Water Controls 58

Appendix-9:Comprehensive Results for K_S, S_t and $\hat{r}$ 72

Appendix-10:Effect of $S_t$on K_S 86

Appendix-11:Biomass Levels Based on Protein Concentrations 88

Appendix-12:Effect of Low EDB Levels on the Rate of 1,2-DCA Dechlorination 90

Appendix-13: GC Response Factors 92

REFERENCES 93

LIST OF TABLES

Table		Page
2.1	Treatments Evaluation for the High Concentration Experiments.	23
A-8.1	Maximum Growth Rates for 1,2-DCA	62
A-8.2	Maximum Growth Rates for EDB	62
A-9.1	Results for K_S, S_t and $\hat{r}$	73
A-10.1	Effect of Including S_t in the Monod Equation on the Fitting Results for K_S	87
A-13.1	GC Response Factors for Volatile Organic Compounds with the FID Detector	92
A-13.2	GC Response Factors for Volatile Organic Compounds with the ECD Detector	92

LIST OF FIGURES

Figure Page

3.1 Representative results for biodegradation of 1,2-DCA in treatment A (***a***); biodegradation of EDB in treatment F (***b***); biodegradation of 1,2-DCA and EDB in treatment B (***c***); and biodegradation of 1,2-DCA and EDB treatment D (***d***). Replicates are shown in Supporting Information. Each arrow indicates addition of 0.31 mmol lactate..... 24

3.2 The data in panel a correspond to the four panels in Figure 3.1; b corresponds to Figure 3.1a and 3.1c; and c is for bottles shown in Supporting Information. Lines represent the model fit. Treatments are described in Table 2.1..... 25

3.3 Summary of average $\hat{\mu}$ (***a***), K_S (***b***), $S_t$values (***c***), and lag times(***d***) for 1,2-DCA and EDB in enrichment cultures grown on 1,2-DCA and EDB. Treatments are defined in Table 2.1. Error bars represent the standard deviation for 6-14 bottles per treatment. 26

3.4 Representative results for the effect of EDB added to the enrichment culture biodegrading high levels of 1,2-DCA with no EDB added (***a***); 370 µg/L EDB added (***b***); and 645 µg/L EDB added (***c***). Each arrow indicates addition of 0.31 mmol lactate. Replicate bottles are shown in Supporting Information. EDB was added on day 16.5 and is shown in log scale (MCL = 0.05 µg/L). 27

3.5 Biodegradation of environmental levels of 1,2-DCA or (and) EDB in an enrichment culture grown with 1,2-DCA as the terminal electron acceptor (***a***) and in an enrichment culture grown with EDB as the terminal electron acceptor

(***b***). Each treatment was done in triplicates. Results for all the individual bottles are shown here. Each arrow indicates addition of 0.25 mmol lactate. 28

A-1.1 Pathways for anaerobic reduction of 1,2-DCA; [H] = H^+ + e^-. The pathways for anaerobic transformation of EDB are identical. 30

A-2.1 Data for 1,2-DCA enrichment culture maintenance. 34

A-2.2 Data for EDB enrichment culture maintenance. 35

A-3.1 Ratio of electron equivalents of lactate added to electron equivalents needed for complete dehalogenation over time (*a*); and the average electron equivalents of lactate added to electron equivalents needed for complete dehalogenation (*b*). The bars in panel b show the maximum and minimum ratios. 38

A-3.2 Lactate additions and organic acid concentrations in four bottles; (*a*) treatment A, bottle #1; (*b*) treatment A, bottle #2; (*c*) treatment F, bottle #1; and (*d*) treatment F, bottle #2. 39

A-4.1 Comparison of different patterns of 1,2-DCA addition; see the text for a description of the feeding strategy for each panel. Arrows indicate lactate additions. 42

A-4.2 Comparison of different patterns of EDB; ; see the text for a description of the feeding strategy for each panel . Arrows indicate lactate additions. 43

A-5.1 Percent recoveries for (*a*) ethene; (*b*) VC, VB and (*c*) methane. Error bars represent the standard deviations based on all bottles in each treatment. 45

A-6.1 Results for treatment A, (*a*) bottle #1; (*b*) bottle #2; (*c*) bottle #3; and (*d*) bottle #4. 47

A-6.2 Results for treatment A, (*a*) bottle #73; (*b*) bottle #74; (*c*) bottle #75; and (*d*) bottle #76. 48

A-6.3 Results for treatment A, (*a*) bottle #A1; (*b*) bottle #A2; and (*c*) bottle #A3. 49

A-6.4 Results for treatment B, (*a*) bottle #24; (*b*) bottle #90; (*c*) bottle #100; (*d*) bottle #109; (*e*) bottle #110; and (*f*) bottle #99 50

A-6.5 Results for treatment C, (*a*) bottle #85; (*b*) bottle #86; (*c*) bottle #87; (*d*) bottle #102; (*e*) bottle #103; and (*f*) bottle #104 51

A-6.6 Results for treatment C, (*a*) bottle #C1; (*b*) bottle #C2; and (*c*) bottle #C3. 52

A-6.7 Results for treatment D, (*a*) bottle #60; (*b*) bottle #105; (*c*) bottle #106; (*d*) bottle #113; (*e*) bottle #93; and (*f*) bottle #94 53

A-6.8 Results for treatment E, (*a*) bottle #60; (*b*) bottle #105; (*c*) bottle #106; (*d*) bottle #113; (*e*) bottle #93; and (*f*) bottle #94 54

A-6.9 Results for treatment E, (*a*) bottle E1; (*b*) bottle #E2; (*c*) bottle #E3. 55

A-6.10 Results for treatment F, (*a*) bottle #42; (*b*) bottle #43; (*c*) bottle #44; (*d*) bottle #101; (*e*) bottle #111; and (*f*) bottle #112 56

A-6.11 Results for treatment F, (*a*) bottle #F1; (*b*) bottle #F2; and (*c*) bottle #F3. 57

A-7.1 Results for the mineral medium controls. 59

A-7.2 Results for the EDB water controls. 60

A-8.1 Results for 1,2-DCA in treatment A, (*a*) bottle #1; (*b*) bottle #2; (*c*) bottle #3; and (*d*) bottle #4. 63

A-8.2 Results for 1,2-DCA in treatment A, (*a*) bottle #73; (*b*) bottle #74; (*c*) bottle #75; and (*d*) bottle #76. 64

A-8.3 Results for 1,2-DCA in treatment B, (*a*) bottle #99; (*b*) bottle #100; (*c*) bottle #109; and (*d*) bottle #110. 65

A-8.4 Results for EDB in treatment B, (*a*) bottle #24; (*b*) bottle #90; (*c*) bottle #10; (*d*) bottle #109; and (*e*) bottle #110. 66

A-8.5 Results for 1,2-DCA in treatment C, (*a*) bottle #85; (*b*) bottle #86; (*c*) bottle #87; (*d*) bottle #102; (*e*) bottle #103; and (*f*) bottle #104. 67

A-8.6 Results for 1,2-DCA in treatment D, (*a*) bottle #96; and (*b*) bottle #94. 68

A-8.7 Results for EDB in treatment D, (*a*) bottle #60; (*b*) bottle #105; (*c*) bottle #106; and (*d*) bottle #113. 69

A-8.8 Results for EDB in treatment E, (*a*) bottle #80; (*b*) bottle #97; (*c*) bottle #98; (*d*) bottle #107; and (*e*) bottle #108. 70

A-8.9 Results for EDB in treatment F, (*a*) bottle #42; (*b*) bottle #43; (*c*) bottle #44; (*d*) bottle #101; (*e*) bottle #111; and (*f*) bottle #112. 71

A-9.1 Results for 1,2-DCA in treatment A, (*a*) bottle #1; (*b*) bottle #2; (*c*) bottle #3; (*d*) bottle #4; (*e*) bottle #73 and (*f*) bottle #74..... 74

A-9.2 Results for 1,2-DCA in treatment A, (*a*) bottle #75; (*b*) bottle #76; (*c*) bottle #A1; (*d*) bottle #A2 and (*e*) bottle #A3..... 75

A-9.3 Results for 1,2-DCA in treatment B, (*a*) bottle #24; (*b*) bottle #90; (*c*) bottle #99; (*d*) bottle #100; (*e*) bottle #109 and (*f*) bottle #110..... 76

A-9.4 Results for EDB in treatment B, (*a*) bottle #24; (*b*) bottle #90; (*c*) bottle #99; (*d*) bottle #100; (*e*) bottle #109 and (*f*) bottle #110. 77

A-9.5 Results for 1,2-DCA in treatment C, (*a*) bottle #85; (*b*) bottle #86; (*c*) bottle #87; (*d*) bottle #102; (*e*) bottle #103 and (*f*) bottle #104..... 78

A-9.6 Results for 1,2-DCA in treatment C, (*a*) bottle #C1; (*b*) bottle #C2 and (*c*) bottle #C3..... 79

A-9.7 Results for 1,2-DCA in treatment D, (*a*) bottle #93 and (*b*) bottle #94. 80

A-9.8 Results for EDB in treatment D, (*a*) bottle #93; (*b*) bottle #94; (*c*) bottle #105 and (*d*) bottle #113. 81

A-9.9 Results for EDB in treatment E, (*a*) bottle #80; (*b*) bottle #97; (*c*) bottle #98; (*d*) bottle #107; (*e*) bottle #108 and (*f*) bottle #E1..... 82

A-9.10 Results for EDB in treatment E, (*a*) bottle #E2 and (*b*) bottle #E3..... 83

A-9.11 Results for EDB in treatment F, (*a*) bottle #101; (*b*) bottle #111; (*c*) bottle #112; (*d*) bottle #F1; (*e*) bottle #F2 and (*f*) bottle #F3. 84

A-9.12 Results for EDB in treatment F, (*a*) bottle #FY1 and (*b*) bottle #FY2. 85

A-11.1 Protein results for (*a*) treatment A, bottle #1; (*b*) treatment A, bottle #2; (*c*) treatment C, bottle #1; (*d*) treatment C, bottle #2; (*e*) treatment E, bottle #1; (*f*), treatment E, bottle #2; (*g*) treatment F, bottle #1; and (*h*) treatment F, bottle #2..... 89

A-12.1 Representative for duplicate bottles showing the effect of EDB added to the enrichment culture biodegrading high levels of 1,2-DCA with no EDB added (*a*); 370 µg/L EDB added (*b*); and 645 µg/L EDB added (*c*). Each arrow indicates addition of 0.31 mmol lactate. Replicate bottles are shown in Figure 3.4. EDB was added on day 16.5 and is shown in log scale (MCL = 0.05 µg/L)..... 91

ABBREVIATIONS

DCA	dichloroethane
EDB	ethylene dibromide (1,2-dibromoethane)
MCL	maximum contaminant level
VB	vinyl bromide
VC	vinyl chloride

CHAPTER ONE

1.0 INTRODUCTION

1.1 Overview

According to the Agency for Toxic Substances and Disease Registry, ethylene dibromide (EDB) is the sixth highest ranked halogenated aliphatic compound on the Priority List of Hazardous Substances,and 1,2-dichloroethane (1,2-DCA) ranks fourteenth. EDB has the second lowest maximum contaminant level (MCL = 0.05 µg/L) in drinking water among all the organic compounds, after dioxin (*2*). The toxicity of 1,2-DCA is comparable to tetrachloroethene and trichloroethene, each with an MCL of 5.0 µg/L(*3*). 1,2-DCA is used as a precursor in the manufacture of vinyl chloride (VC) (*4*). EDB was widely used as a pesticide and soil fumigant until these applications were banned in the 1980s. 1,2-DCA and EDB are among the most frequently detected contaminants in drinking water when an MCL is exceeded(*5*). 1,2-DCA has been found at 570 National Priorities List sites (*3*), while EDB has been detected at 27 sites (*2*).

The co-occurrence of EDB and 1,2-DCA in groundwater results mainly from environmental releases of leaded gasoline; these compounds were added as lead scavengers. Even though leaded gasoline has not been used for several decades, Falta et al. (*6*) demonstrated that EDB and 1,2-DCA have persisted at many sites that once had leaking storage tanks. Based on an evaluation of 1100 sites in South Carolina, 537 had EDB levels higher than its MCL. The predicted maximum concentrations of EDB and 1,2-DCA in groundwater from a leaded gasoline release are 1.9 and 3.7 mg/L, respectively (*7*). The co-occurrence of EDB and 1,2-DCA has also been reported for at least one industrial site, where concentrations of 1,2-DCA and EDB in the vicinity of the source area were approximately 100 mg/L and 10 mg/L, respectively (*8*).

Under low redox anaerobic conditions, EDB and 1,2-DCA can be used as terminal electron acceptors via organohalide respiration. The predominant pathway is dihaloelimination directly to ethene (Appendix-1). Hydrogenolysis yields minor amounts of bromoethane from EDB and chloroethane from 1,2-DCA, both of which may be further reduced to ethane. Dehydrohalogenation yields minor amounts of vinyl bromide (VB) from EDB and VC from 1,2-DCA, both of which may undergo hydrogenolysis to ethene. Three types of microbes have been shown to use 1,2-DCA as a terminal electron acceptor, including *Dehalococcoides* (*9-15*), *Dehalobacter* (*13, 16*)and *Desulfitobacterium* (*17-21*). Use of EDB as a terminal electron acceptor has been demonstrated only with *Dehalococcoides ethenogenes*strain195 (*10, 15*). Hydrogen serves as the electron donor.

No information was found in the literature on Monod kinetic parameters (maximum specific growth rate and half saturation coefficient) for use of 1,2-DCA or EDB as terminal electron acceptors. First order degradation rates have been reported, ranging from 0.44-18 yr^{-1} for 1,2-DCA and 1.5-110 yr^{-1} for EDB (*22*). Wilson et al. (*23*) estimated a half saturation coefficient for EDB of 490-1000 μg/L based on the similarity of its solubility to VC; however, experimental measurements were not made. In addition to a lack of information on the kinetics of 1,2-DCA and EDB utilization, no studies were found that evaluated the effect of each compound on the rate of biodegradation of the other, when they are present together. A microcosm study by Henderson et al. (*1*) with soil and groundwater from a site contaminated with leaded gasoline demonstrated anaerobic biodegradation of EDB at a higher rate, and to a greater extent, than 1,2-DCA. However, it was not clear if EDB inhibited use of 1,2-DCA, or if it was simply consumed at a higher rate. At an industrial site where EDB and 1,2-DCA were present at levels above 10 mg/L, reductive dehalogenation of both compounds was observed (*8*).

However, there was no indication that the co-occurrence of 1,2-DCA and EDB had an effect on the rate of dehalogenation of either compound.

1.2ResearchObjectives

The objectives of this study were to measure maximum specific growth rates, half saturation coefficients, and lag times in enrichment cultures that use 1,2-DCA and EDB as terminal electron acceptors; and to evaluate if the presence of EDB has an effect on the kinetics of 1,2-DCA dehalogenation, and vice versa. The effect of each compound on biodegradation rates of the other was evaluated at the high concentrations that may be found at industrial sites (e.g., >10 mg/L) and at the lower concentrations that have been reported at leaded gasoline sites.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Chemicals and Media

The purity and sources of chemicals used are as follows: EDB (99%) from Acros Organics; 1,2-DCA (99%) from Mallinckrodt; VC (99.5%) from Fluka; polymer grade ethene (99.9%) from Airgas; ethane (99.95%) and methane (99%) from Matheson; bromoethane (99%) from EMD Chemicals; chloroethane (99.7%) from Sigma-Aldrich; and VB (98%) from Pfaltz & Bauer. Sodium lactate syrup was obtained from EM Science (containing 58.8-61.2% sodium lactate; specific gravity = 1.31). All other chemicals were reagent grade.

The mineral salts medium used to grow the enrichment cultures had the following constituents per liter of distilled, deionized water: 10 mL of phosphate buffer (52.5 g of K_2HPO_4 per liter), 10 mL of salt solution (53.5 g of NH_4Cl per liter, 4.7 g $CaCl_2 \cdot 2H_2O$ per liter, 1.8 g of $FeCl_2 \cdot H_2O$ per liter), 2 mL of trace mineral solution (0.3 g of H_3BO_3 per liter, 0.2 g of $ZnSO_4 \cdot 7H_2O$ per liter, 0.75 g of $NiCl_2 \cdot 6H_2O$ per liter, 1.0 g of $MnCl_2 \cdot 4H_2O$ per liter, 0.1 g of $CuCl_2 \cdot 2H_2O$ per liter, 1.5 g of $CoCl_2 \cdot 6H_2O$ per liter, 0.02 g of Na_2SeO_3 per liter, 0.1 g of $Al_2(SO_4)_3 \cdot 16H_2O$ per liter, 1 mL of concentrated HCl per liter), 2 mL of $MgSO_4 \cdot 7H_2O$ solution (62.5 g/L), 1 mL of resazurin solution (1.0 g/L), 50 mL of filter-sterilized $NaHCO_3$ solution (16.0 g/L), 10 mL of filter-sterilized yeast extract solution (5.0 g/L), 0.24 g of $Na_2S \cdot 9H_2O$ and 0.1448 g of $FeCl_2 \cdot H_2O$. The bicarbonate and yeast extract solutions were added after the medium was autoclaved and cooled. The container was then placed in an anaerobic chamber where the sulfide and ferric chloride were added. After dispensing the medium to serum bottles, they were removed from the chamber and the headspaces were purged with 70% N_2/30% CO_2. The pH was adjusted to approximately 7.0 with 8 M NaOH.

2.2Analytical Methods

Volatile organic compounds were monitored by headspace analysis (*24*). 1,2-DCA, EDB, chloroethane, bromoethane, VC, VB, ethane, ethene, and methane were evaluated using a Hewlett-Packard 5890 Series II Gas Chromatograph, equipped with a flame ionization detector in conjunction with a column packed with 1% SP-1000 on 60/80 Carbopack-B (Supelco, Inc.). Nitrogen was used as the carrier gas. The temperature program was 60°C for 2 min, ramp at 20°C/min to 150°C, ramp at 10°C/min to 185°C and hold for 5 min. Detection limits ranged from 0.2 to 6.0 µg/L for the halogenated compounds except for EDB, which had a detection limit of 23 µg/L. To quantify EDB at lower concentrations, headspace samples were analyzed using a Hewlett-Packard 5890 Series II Plus Gas Chromatograph equipped with an electron capture detector, as previously described (*1*). The detection limit for EDB with this method was 0.05 µg/L. Different syringes were used for the flame ionization detector and electron capture detector measurements of EDB to minimize the potential for carry-over of compound adsorbed to the syringe.

The gas chromatograph response to a headspace sample (0.5 mL) was calibrated to give the total mass of the compound (*M*) in that bottle (*24*). Assuming the headspace and aqueous phases were in equilibrium, the total mass present was converted to an aqueous-phase concentration with the following equation:

$$C_l = \frac{M}{V_l + H_C V_g} \tag{2.1}$$

where $C_l$is the concentration in the aqueous phase (in micromolar units), M is the total mass present (µmol/bottle), $V_l$is the volume of the liquid in the bottle (L), $V_g$is the volume of the headspace in the bottle (L), and $H_C$is the Henry's law constant (gas concentration [mol/m^3]/aqueous concentration [mol/m^3]) at 23°C for 1,2-DCA (*25*), EDB (*25*), VC (*24*),

choroethane (*24*), ethene (*26*), and ethane (*26*), and at 25°C for methane (*27*), bromoethane (*27*), and VB (*28*).

Protein was measured after lysing samples of the enrichment cultures, using a Compat-Able™ assay preparation reagent set and a BCA™ protein assay kit (Pierce Chemical Company), following the manufacturer's enhanced protocol (*29*).

2.3 Enrichment Cultures

Two anaerobic enrichment cultures were developed; one was provided with 1,2-DCA as the sole terminal electron acceptor and the other with EDB. The inoculum for both cultures was an enrichment culture that uses all of the chlorinated ethenes as terminal electron acceptors, with *Dehalococcoides* yields ranging from 6.8×10^5 to 1.8×10^9 gene copies/μmol Cl^- or Br- released (*30*). When provided with 1,2-DCA or EDB, the enrichment started dehalogenating these compounds at increasing rates. Sodium lactate was used as the electron donor for both cultures. Routine monitoring data for the enrichment cultures over 16-26 months is provided in Supplementary Information (Appendix-2), along with maintenance procedures. Ethene was the principal product from 1,2-DCA and EDB; trace levels of VC and VB were also present. Sodium hydroxide was added to keep the pH between 6.7 and 7.1.

2.4Batch Kinetics at High Concentrations

For the purposes of this study, 9.2-110 mg/L of 1,2-DCA and 17-86 mg/L of EDB were considered to be high concentrations. The six treatments evaluated for the high concentration experiments are summarized in Table 2.1. Two received 1,2-DCA, two received EDB, and two

received a mixture. One half of the treatments were inoculated with the 1,2-DCA enrichment culture, the others with the EDB enrichment culture.

Kinetic coefficients were determined in batch tests in 160 mL serum bottles. Experiments were initiated in an anaerobic chamber by combining 2 mL from one of the enrichment cultures with 98 mL of medium. The serum bottles were then sealed with Teflon-faced red rubber septa and crimp caps. Outside the chamber the headspaces were purged (70% N_2/30% CO_2), the bottles were resealed, and the pH was adjusted (with NaOH) to 6.9-7.1. Serum bottles were incubated on a shaker table at room temperature (22-24°C) to provide continuous mixing, with the liquid in contact with the septa. Lactate was added so that the initial ratio of electron equivalents of donor to equivalents needed for dehalogenation was higher than 10. Repeated additions of lactate were made to ensure it remained in excess throughout the incubation period; acetate and propionate were the principal organic acids that accumulated (Appendix-3).

The initial amount of 1,2-DCA added (using a water saturated solution or neat compound) was 9.6-26 μmol/bottle (9.2-25 mg/L). When it became apparent that a rapid rate of consumption had begun, repeated additions of 1,2-DCA were made in order to maintain saturation conditions. The highest level of 1,2-DCA reached was 115 μmol/bottle (110 mg/L). Once the rate of 1,2-DCA consumption reached a maximum, no more was added and the rate of consumption was monitored until it decreased either to a transition level or below the detection limit. The initial amount of EDB added was 9.1-21 μmol/bottle (17-39 mg/L). The maximum amount reached was 46.8 μmol/bottle (86.2 mg/L); this was less than the maximum amount of 1,2-DCA, since higher concentrations of EDB became inhibitory. This pattern of adding 1,2-DCA and EDB was adopted after trying other strategies, including addition of a single large dose at the outset. However, especially with EDB, initial concentrations above approximately 100

mg/L were inhibitory (Appendix-4). By starting at a lower initial concentration and increasing the amounts added over time, the cultures were able to acclimate.

Batch experiments were performed in triplicate and were repeated at least three times, over a 17-month period. Of these, at least two bottles for each treatment exhibited high rates of EDB and/or 1,2-DCA consumption.

Maximum growth rates ($\hat{\mu}$, d^{-1}) based on 1,2-DCA and EDB consumption were determined using a method adapted from the respirometic procedure of Brown et al. (*31*). Assuming that endogenous decay is negligible during periods of exponential growth, the rate of substrate utilization can be calculated as:

$$-\frac{dS}{dt} = \frac{X}{Y} \cdot \frac{\hat{\mu} S}{K_s + S} \tag{2.2}$$

where S is the substrate concentration (mg/L), Y is the yield (mg biomass/mg 1,2-DCA or EDB), X is the biomass concentration (mg/L), and K_S is the half saturation coefficient (mg/L of 1,2-DCA or EDB). In this study, maximum growth rates were determined based on the cumulative amount of terminal electron acceptor consumed (i.e., 1,2-DCA and EDB), or S_u(mg 1,2-DCA or EDB per liter). X can be expressed as the initial biomass concentration (X_o) plus the amount formed based on the yield (expressed in terms of electron acceptor consumption). Making these substitutions, equation 2.2 becomes:

$$\frac{dS_u}{dt} = \frac{(X_o + S_u Y)}{Y} \cdot \frac{\hat{\mu} S}{K_s + S} \tag{2.3}$$

Under batch conditions, when the substrate concentration is high relative to the half saturation constant (i.e., $S >> K_S$) and the initial biomass concentration is low relative to the new amount formed (i.e., $S_u Y >> X_o$), equation 2.3 simplifies to:

$$\frac{dS_u}{dt} = \hat{\mu} S_u \tag{2.4}$$

Separating variables and integrating equation 2.4 results in:

$$\ln S_u = \hat{\mu} t \tag{2.5}$$

Maximum growth rates were determined from the slope for equation 2.5, based on the cumulative amount of EDB and 1,2-DCA consumed during the exponential phase of activity. Averages and standard deviationswere calculated based on the results from 2-8 serum bottles per treatment.

Monod half saturation coefficients (K_S), transition concentrations (S_t, mg 1,2-DCA or EDB per liter), and maximum substrate consumption rates ($\hat{r}$, mg 1,2-DCA or EDB per liter per day) were determined in the same serum bottles used to measure the maximum growth rates. Following the final addition of EDB or 1,2-DCA, the rate of consumption was monitored until the level reached a transition concentration or decreased below detection. Based on similar batch depletion studies with VC, mass transfer between the headspace and liquid phases was assumed not to be limiting, so that monitoring of the headspace represented the aqueous phase concentrations for all of the halogenated volatile compounds (*32*). Sufficient amounts of 1,2-DCA and EDB were consumed to ensure that the biomass levels reached steady state, i.e., so that the growth rate equaled the decay rate. This was confirmed by protein measurements (Appendix-11).

Since biomass concentrations reached a maximum ($\hat{X}$) and $\hat{\mu}/Y$ is a constant (i.e., the maximum specific substrate removal rate), then the maximum reaction rate ($\hat{r}$) is:

$$\hat{r} = \frac{\hat{\mu}}{Y} \hat{X} \tag{2.6}$$

Substituting equation 2.6 into equation 2.2 yields:

$$-\frac{dS}{dt} = \frac{\hat{r} \cdot S}{K_s + S} \tag{2.7}$$

In nearly all of the bottles that received high doses of 1,2-DCA and/or EDB, the rate of consumption slowed down faster than what is predicted by equation 2.7. At this transition point, EDB and 1,2-DCA levels reached a plateau or decreased at a considerably slower rate. To account for this behavior, equation 2.7 was modified to include a transition concentration (S_t):

$$-\frac{dS}{dt} = \frac{\hat{r} \cdot (S - S_t)}{K_s + (S - S_t)} \tag{2.8}$$

K_S, S_t and $\hat{r}$ were determined by a weighted, nonlinear least-squares method by fitting of batch depletion data for 1,2-DCA and EDB to equation 2.8 using the software AQUASIM (*32-33*). Each data point was weighted with the inverse of the substrate concentration. The fitting process was initiated with the simplex optimization method and then fully optimized by the secant method, which reports the standard deviation of each parameter(*32*). Equation 2.8 was fit to data from each serum bottle to obtain initial estimates of K_S, S_t and$\hat{r}$. Data from all bottles within a treatment were then pooled and, using the average results from individual bottles as a starting point, equation 2.8 was fit simultaneously to determine K_S and S_t; values for $\hat{r}$ remained bottle-specific.

The lag time for each bottle was the time interval from day zero till the onset of exponential growth; averages and standard deviations were calculated based on the lag times in individual bottles.

2.5Biodegradation of 1,2-DCA and EDB at Concentrations Representative of Leaded Gasoline Spills

For the purposes of this study, 1,2-DCA and EDB concentrations less than or equal to 4.5 mg/L and 2.2 mg/L, respectively, were considered to be representative of the levels found near the source zones at leaded gasoline releases (*7*). Separate experiments were performed at these lower concentrations in the same manner as described above, except that only a single addition of 1,2-DCA and/or EDB was made. Lactate was maintained in excess.

CHAPTER THREE

3.0 RESULTS

3.1 High Concentrations of 1,2-DCA and EDB

Representative results for the batch experiments are shown in Figure 3.1. The y-axes are expressed in μmol per bottle, in order to directly reveal the stoichiometry of the parent compounds and daughter products. Consumption of 1,2-DCA (initial amount = 21.6 μmol/bottle, or an aqueous phase concentration of 20.7 mg/L) in treatment A (inoculated with the 1,2-DCA enrichment culture; Fig. 3.1a) started after a lag period of 10 days. Ethene was the predominant product, amounting to 76.1 ± 7.2% of the 1,2-DCA consumed; the highest average VC accumulation was 0.61% of the 1,2-DCA consumed (Appendix-5). Methanogenesis was partially inhibited until 1,2-DCA levels decreased to below approximately 5 μmol/bottle (4.8 mg/L). Consumption of EDB (initial amount = 10.2 μmol/bottle, or an aqueous phase concentration of 18.8 mg/L) in treatment F (inoculated with the EDB enrichment culture; Fig. 3.1b) started after a lag period of approximately 5 days. Ethene was the predominant product, amounting to 75.6 ± 9.2% of the EDB consumed; the highest average VB accumulation was

0.052% of the EDB consumed (Appendix-5). Methanogenesis was suppressed for the duration of the test. Results for 1,2-DCA inoculated with the EDB enrichment culture (treatment C) and EDB inoculated with the 1,2-DCA enrichment culture (treatment E) were similar to those shown in Figure 3.1a and 3.1b, respectively (Appendix-6). This indicated that the 1,2-DCA enrichment culture had no difficulty in switching to EDB as the terminal electron acceptor, and vice versa.

For treatment B, in which 1,2-DCA and EDB were added together and inoculated to the 1,2-DCA culture, EDB consumption started first, following a lag period of approximately 10 days (Fig. 3.1c). 1,2-DCA consumption did not start until EDB decreased to close to its detection limit. The pattern of EDB and 1,2-DCA additions was similar to those used when the individual compounds were added (Fig. 3.1a and 3.1b). Ethene was the predominant product of both compounds; the highest average VB accumulation was 0.072% of the total EDB consumed and the highest average VC accumulation was 0.40% of the 1,2-DCA consumed (Appendix-5). Methanogenesis was inhibited throughout the entire incubation period.

Similar results were obtained for the treatment D, in which 1,2-DCA and EDB were added simultaneously and the EDB enrichment culture was used as the inoculum (Fig. 3.1d). EDB consumption started first, following a lag period of approximately 10 days. 1,2-DCA consumption started approximately 45 days after EDB decreased below 15 µg/L. Ethene was the predominant product of both compounds; the highest average VC accumulation was 0.64% of the 1,2-DCA consumed and the highest average VB accumulation was 0.093% of the EDB consumed (Appendix-5). Methanogenesis was only inhibited by the presence of EDB.

Water and medium control results demonstrated that the consumption of 1,2-DCA and EDB in the serum bottles was due to biotic processes. There was no statistically significant loss of either compound over incubation periods similar to those shown in Figure 3.1 (Appendix-7).

Based on results from the time periods when there was an exponential increase in the rate of 1,2-DCA and EDB consumption, the data were fit to equation 2.5 to determine the maximum growth rates. Representative results are shown in Figure 3.2a for the same bottles shown in Figure 3.1 (complete results are in Appendix-8) and average results for all treatments are shown in Figure 3.3a. The maximum growth rate on 1,2-DCA was equivalent for the treatments inoculated with the 1,2-DCA enrichment culture, with or without EDB added (A and B, respectively), and in the treatment inoculated with the EDB culture (treatment C). The treatment inoculated with the EDB culture that received EDB and 1,2-DCA together had a lower growth rate on 1,2-DCA (treatment D) (Scheffe's test, α=0.05), suggesting that prior growth on EDB slowed the subsequent rate of growth on 1,2-DCA. However, it should be noted that only two bottles were evaluated for treatment D, compared to 4-8 bottles for treatments A, B, and C. There was no significant difference among the maximum growth rates for EDB, i.e., B, D, E and F (Fig. 3.3a) (Scheffe's test, α=0.05). Overall, the average growth rate on 1,2-DCA for all treatments (0.50 d^{-1}) was significantly higher than that for EDB (0.38 d^{-1}) (Student's *t*-test, α=0.05).

Figure 3.2b and 3.2c show representative batch depletion data that were fit to equation 2.8, providing K_S and S_t. During this period of incubation, protein concentrations did not change (Appendix-11), consistent with the assumption that biomass levels remained constant over this interval. Figure 3.2b shows the model fit for the treatment with 1,2-DCA as the electron acceptor, corresponding to Figure 3.1a and 3.1c. Figure 3.2c is for bottles that received EDB as the electron acceptor; results for the full duration of the incubation period for these bottles are in Appendix-6. Average results for K_S are presented in Figure 3.3b; the values for 1,2-DCA are an order of magnitude higher than for EDB. When both compounds were added, the Ks value for

1,2-DCA increased significantly, suggesting an inhibitory caused effect for EDB. The lowest K_S value for EDB occurred when only EDB was added and the 1,2-DCA enrichment culture served as the inoculum (treatment E), while the highest was for EDB alone and the EDB culture was the inoculum (treatment F). The presence of 1,2-DCA along with EDB did not increase the K_S for EDB when the 1,2-DCA culture was the inoculum (i.e., treatment B versus E), although the standard deviations exceed the averages. The presence of 1,2-DCA along with EDB decreased the K_S for EDB when the EDB culture was the inoculum (i.e., treatment D versus F), suggesting that 1,2-DCA had a stimulatory effect.

Similar to K_S, the transition levels for 1,2-DCA were one to two orders of magnitude higher compared to EDB (Fig. 3.3c.). The most notable influence on S_t for 1,2-DCA occurred in the treatment with both compounds added and inoculated with the EDB enrichment culture (treatment D), which was significantly higher than when only 1,2-DCA was added (treatment C). With EDB, the standard deviations for S_t were higher. Nevertheless, the transition level was significantly lower for EDB alone when the EDB culture was the inoculum (treatment F) versus the 1,2-DCA culture (treatment E) (Student's *t*-test, α=0.05). For most of the treatments, once the transition level was reached, little or no additional dehalogenation occurred. However, 18% of the bottles in treatments A and 33% in treatment C did eventually reach the MCL for 1,2-DCA; 25% of the bottles in treatment F eventually reached MCL for EDB.

The maximum substrate utilization rates ($\hat{r}$) for 1,2-DCA and EDB were 12.7 (±5.3) and 13.9 (±5.8) mg/L/d, respectively (Appendix-9). The coexistence of 1,2-DCA and EDB did not significantly change $\hat{r}$ for either compound, with one exception: When the EDB enrichment culture served as the inoculum, the presence of EDB decreased the $\hat{r}$ for 1,2-DCA by 45% (Student's *t*-test, α=0.05).

The presence of EDB with 1,2-DCA significantly increased the lag times for 1,2-DCA (i.e., treatment A versus B and C versus D; Fig. 3.3d). In addition, the lag time for 1,2-DCA was longer in the treatments inoculated with the EDB enrichment culture (i.e., treatment A versus C and B versus D). Conversely, the lag time for EDB was not impacted by the presence of 1,2-DCA (regardless of the inoculum) and was equivalent to the lag time for 1,2-DCA in treatment A.

3.2Effect of Low EDB Levels on 1,2-DCA

The results in Figure 3.1c and 3.1d indicated that biodegradation of 1,2-DCA was inhibited until EDB levels decreased close to the detection level. To verify this phenomenon, three sets of duplicate serum bottles were prepared with 1,2-DCA as the sole substrate and the 1,2-DCA enrichment culture as inoculum (2% v/v), in the same manner described for the batch kinetic tests. All bottles received three additions of 1,2-DCA; Figure 3.4 shows what occurred during the third addition. The y-axes on Figure 3.4 are in terms of aqueous phase concentrations, since daughter products are not shown. A comparatively small amount of EDB was added to two of the sets (i.e., 370 µg/L EDB in Fig. 3.4b and 645 µg/L in Fig. 3.4c) when the 1,2-DCA amount remaining had decreased by 32-45%. Addition of these low levels of EDB immediately decreased the rate of 1,2-DCA consumption, while the EDB was consumed at a high rate. As the EDB level decreased close to its MCL, a high rate of 1,2-DCA consumption resumed. This demonstrated that EDB was used by the cultures to the exclusion of 1,2-DCA. There was no apparent impact of the short-term exposure to EDB on the subsequent rate of 1,2-DCA consumption.

3.3Biodegradation of 1,2-DCA and EDB at Concentrations Representative of Leaded Gasoline Spills

Figure 3.5 presentsthe results for batch tests started at 1,2-DCA and EDB concentrations similar to those found near the source zone of leaded gasoline spills. With the 1,2-DCA enrichment culture as inoculum, EDB was consumed first, reaching its MCL in 11-22 days (Fig. 3.5a). The presence of 1,2-DCA along with EDB had mixed effects on the rate of EDB biodegradation; in two of the three replicates, bottles with 1,2-DCA reached the MCL for EDB first, while in one of the bottles EDB consumption was slower than in the bottles with only EDB present. In the treatment with only 1,2-DCA, the lag time was approximately 9-14 days before the onset of biodegradation, with the MCL reached by day 28-45. With EDB present, biodegradation of 1,2-DCA started shortly after the EDB reached its MCL and the 1,2-DCA was consumed at an equivalent or slightly faster rate than in the treatment with 1,2-DCA alone, suggesting that prior exposure to EDB had a slightly beneficial impact.

With the EDB enrichment culture as inoculum, EDB was consumed first, in the presence or absence of 1,2-DCA (Fig. 3.5b). The treatment with EDB alone reached the MCL first; however, the time required to reach the MCL was notably longer (34-38 days) than with the 1,2-DCA enrichment culture. The lag time prior to the onset of 1,2-DCA biodegradation was 49-61 days, considerably longer than for the 1,2-DCA enrichment culture (Fig. 3.5a). In the treatment with both compounds present, biodegradation of 1,2-DCA started around the time when EDB reached its MCL. Overall, lag times for the onset of 1,2-DCA and EDB biodegradation at the lower initial concentrations were equivalent to or shorter than at the concentrations used in the higher concentration kinetic tests (Fig. 3.3c).

CHAPTER FOUR

4.0 DISCUSSION

The results of this study provide maximum specific growth rates and half saturation coefficients for 1,2-DCA and EDB, when the compounds were available individually and in mixtures, using enrichment cultures that were grown on each compound. EDB was used in preference to 1,2-DCA when both compounds were present. This is consistent with the observation made by Henderson et al. (*34*) based on a microcosm study of a site contaminated with leaded gasoline. The free energy change for EDB dehalogenation to ethene is slightly better than for 1,2-DCA, although the magnitude of the difference (6.7 kJ/mole) is not necessarily a compelling explanation for the preference of debromination over dechlorination (*34*). Regardless, reductive debromination is more favorable than dechlorination for other types of halogenated organics, not just dihaloethanes. For example, polybrominated biphenyls are used in preference to polychlorinated biphenyls (*35*). This preference was especially apparent when dechlorination of 1,2-DCA was interrupted by adding EDB at a concentration more than 100 times lower than the 1,2-DCA; use of 1,2-DCA did not resume until the EDB decreased close to its MCL (Fig. 3.4).

The maximum specific growth rates for 1,2-DCA ranged from 0.19 to 0.58 d^{-1}, which is similar to the 0.21 d^{-1} rate for *Desulfitobacterium dichloroeliminans* strain DCA1, based on its yield and maximum utilization rate (*21*). The maximum specific growth rates for EDB ranged from 0.30 to 0.45 d^{-1}; no previous studies were found that measured $\hat{\mu}$ for EDB. Similar growth rates have been reported for *cis*-1,2-dichloroethene (e.g., 0.17-0.26 d^{-1}, calculated from the yield and maximum utilization rate reported by Yu et al. (*36*)). A number of studies report maximum dechlorination rates for 1,2-DCA; e.g., 150 µM/d for an enrichment culture containing

predominantly *Dehalobacter*(*16*), 630 µM/d for *Dehalococcoides ethenogenes* strain 195 (*9*), and 1300 µM/d for an enrichment containing *Desulfitobacterium dichloroeliminans* strain DCA1 (*21*). The 1,2-DCA transformation rate observed during this study ($\hat{r}$) was 130 µM/d. A maximum debromination rate of 80 µM/d for EDB was estimated from Tandoi et al. (*37*); this is similar to the rate observed in this study (74 µM/d).

No previous studies were found that reported K_S values for 1,2-DCA or EDB. In general, the values measured in this study for 1,2-DCA (58-158 µM) are notably higher than what has been reported for polychlorinated ethenes (e.g., 1.6-3.9 µM for tetrachloroethene, 1.8-2.8 µM for trichloroethene, 1.8-1.9 µM for *cis*-dichloroethene) but similar in magnitude to VC (63-602 µM), as reported by Yu et al. (*38*). The K_S values measured for EDB are considerably lower, with three of the four treatments at or below 0.082 µM. Nevertheless, the K_S for EDB is two orders of magnitude higher than its MCL (0.00027 µM).

Use of a transition concentration (S_t) in the Monod substrate depletion equation allowed for a better fit of the model at low concentrations of 1,2-DCA and EDB. During the experiments to determine $\hat{\mu}$ and K_S, the rate of 1,2-DCA and EDB consumption slowed or stopped in a majority of the bottles before the MCL was reached. S_t levels ranged from 12.9 to 128 µg/L for 1,2-DCA and 0.50 to 9.2 µg/L for EDB. When the 1,2-DCA and EDB depletion date were fit to equation 2.7 (i.e., without S_t), there was a notable increase in the error associated with K_S, while the value of K_S for 1,2-DCA did not change substantially or increased somewhat; the K_S value for EDB was unchanged for treatment F and increased 2.6 to 336-fold for treatments B, D and E when S_t was not included (Appendix-10). Thus, inclusion of S_t had a notably greater influence on the K_S for EDB.

Coleman et al. (*39*) used a similar modification to an equation for predicting oxygen consumption during aerobic biodegradation of VC, although in their experiments a true threshold existed. In our study, S_t was not a true threshold, since 1,2-DCA and EDB levels continued to decrease at a slow rate in some instances. The onset of a slower rate of consumption and/or the cessation of dehalogenation activity appeared to be a consequence of the high concentrations of 1,2-DCA and EDB that were added, since in the lower concentration experiment, all of the replicates reached the MCL for both compounds (Fig. 3.5). This suggests that reaching the MCL may not reliably occur in the vicinity of a source zone for a release of neat material. However, as the concentration decreases away from the source area, the likelihood of reaching the MCL should improve, provided that the system remains anaerobic.

One of the concerns with cultures that dechlorinate 1,2-DCA is the potential for accumulation of VC. In this study, VC accumulation reached 7.6 µM (0.40-0.68% of the total 1,2-DCA consumed), although subsequent decreases in VC occurred with continuing incubation (Fig. 3.1). No accumulation of VC was reported in laboratory studies with *Desulfitobacterium dichloroeliminans* strain DCA1 (*20*-*21*)), although 20-30 µM accumulation occurred in a pilot-scale bioaugmentation test (*17*). VB accumulation was lower than VC (1.3 µM, or 0.05-0.09% of the total EDB consumed) and it too decreased over time (Fig. 3.1).

Evidence continues to accumulate for the need to monitor 1,2-DCA and EDB contamination of groundwater, especially at formed leaded-gasoline sites (*23*). Corresponding interest in remediation approaches is likely to increase. Bioaugmentation is a candidate approach for sites where monitored natural attenuation is infeasible. Although considerable information is available on cultures that can dechlorinate 1,2-DCA, most have not been tested for their ability to debrominate EDB. Of the two enrichment cultures evaluated in this study, the 1,2-DCA culture

has the advantage of more rapid transition to 1,2-DCA after EDB is consumed (Fig. 3.5). Additional information is needed on the ability of enrichment cultures to dehalogenate 1,2-DCA and EDB in the presence of persistent fuel hydrocarbons.

CHAPTER FIVE

5.0 CONCLUSIONS

Based on the results of this study, the following conclusions were reached:

- The cultures enriched with either EDB or 1,2-DCA were able to use either compound as a terminal electron acceptor. Biodegradation occurred predominantly via dihaloelimination to ethene.
- Maximum specific growth rates ($\hat{\mu}$) ranged from 0.19 to 0.58 $d^{-1}$for 1,2-DCA and from 0.30 to 0.45 d^{-1} for EDB.
- Maximum transformation rates were 130 µM/d for 1,2-DCA and 74 µM/d for EDB.
- The half saturation coefficient for 1,2-DCA (5.7-15.7 mg/L, or 58-158 µM) was considerably higher than the K_S values for EDB, with three of the four treatments at or below 15 µg/L (0.082 µM).
- The inhibitory effect of EDB on 1,2-DCA was demonstrated. In treatments in which both compounds were present, EDB was always consumed first and adversely impacted the kinetics of 1,2-DCA utilization. In separate experiments with 1,2-DCA provided alone, dechlorination of 1,2-DCA was interrupted by adding EDB at a concentration more than 100 times lower than the remaining 1,2-DCA; use of 1,2-DCA did not resume until the EDB decreased close to its MCL.
- Experiments performed on low concentrations of EDB and 1,2-DCA (representative of environmental levels at leaded gasoline spills) demonstrated that the culture enriched on 1,2-DCA had better potential for bioaugmentation. Compared with the EDB enrichment culture, the 1,2-DCA culture had the advantage of more rapid transition to 1,2-DCA after EDB was consumed.

Overall, the results of this study contribute to a general understanding of dehalogenation of chlorinated and brominated compounds.

TABLE

Table 2.1 Treatments Evaluation for the High Concentration Experiments.

Treatment	Compound(s) Added	Inoculum
A	1,2-DCA	1,2-DCA EC[a]
B	1,2-DCA + EDB	1,2-DCA EC
C	1,2-DCA	EDB EC
D	1,2-DCA + EDB	EDB EC
E	EDB	1,2-DCA EC
F	EDB	EDB EC

[a] EC = enrichment culture

FIGURES

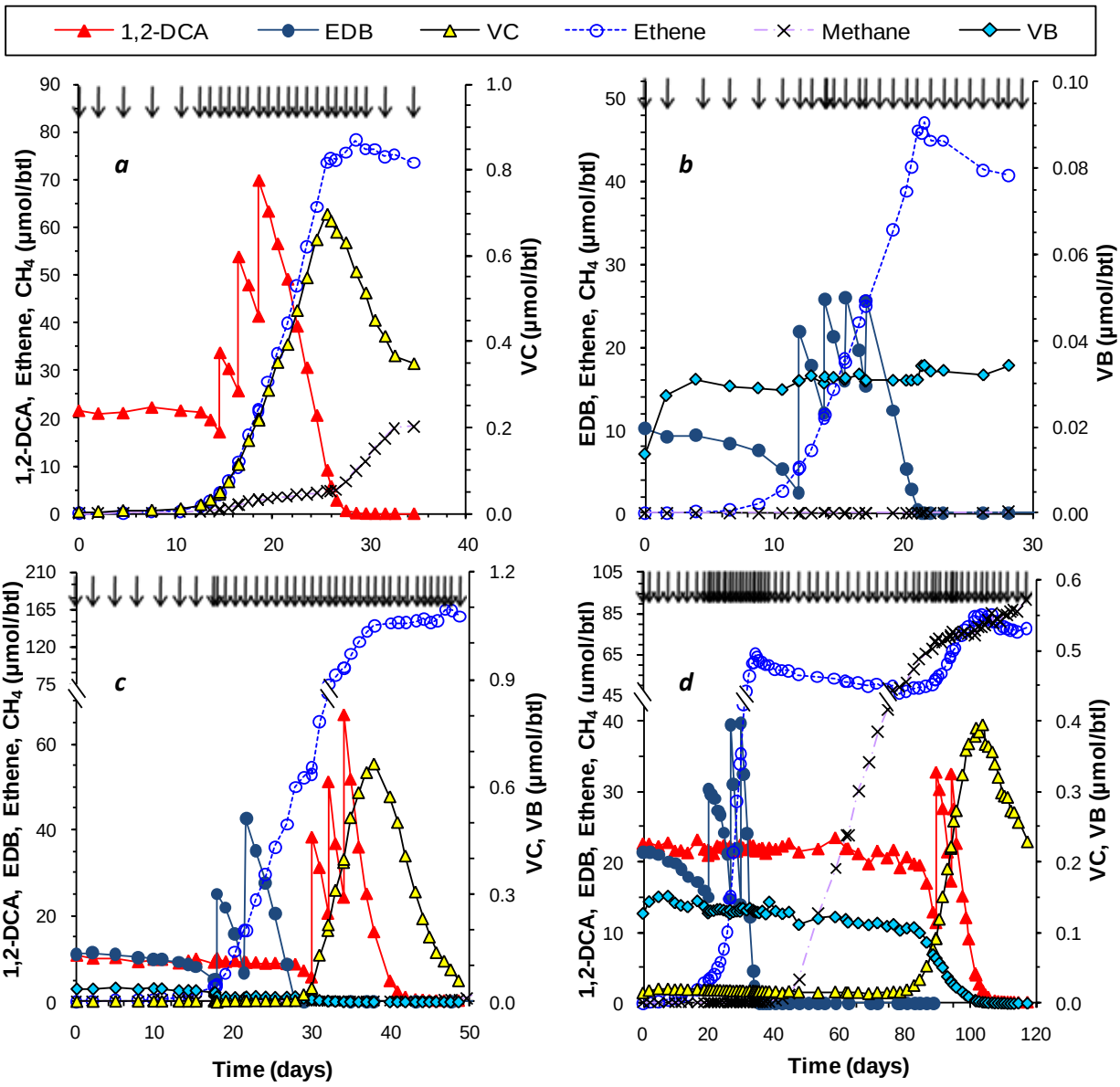


Figure 3.1 Representative results for biodegradation of 1,2-DCA in treatment A (***a***); biodegradation of EDB in treatment F (***b***); biodegradation of 1,2-DCA and EDB in treatment B (***c***); and biodegradation of 1,2-DCA and EDB treatment D (***d***). Replicates are shown in Supporting Information. Each arrow indicates addition of 0.31 mmol lactate.

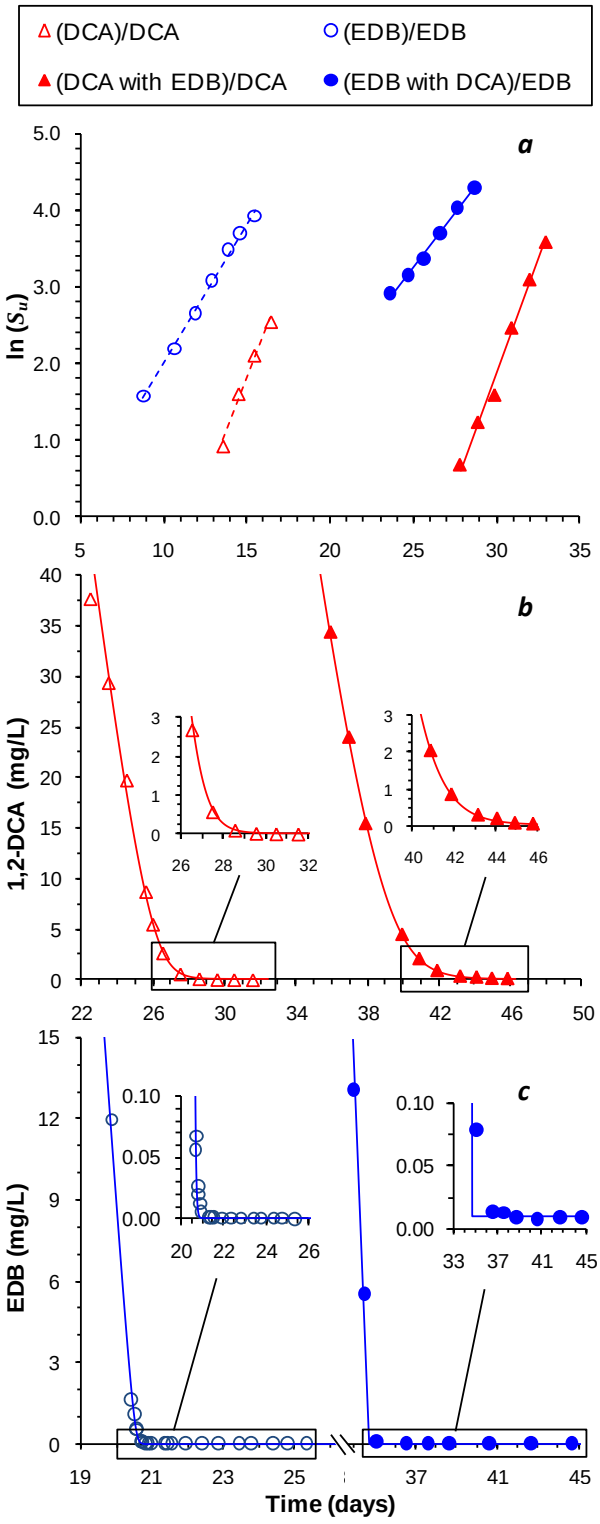


Figure 3.2 The data in panel a correspond to the four panels in Figure 3.1; b corresponds to Figure 3.1a and 3.1c; and c is for bottles shown in Supporting Information. Lines represent the model fit. Treatments are described in Table 2.1.

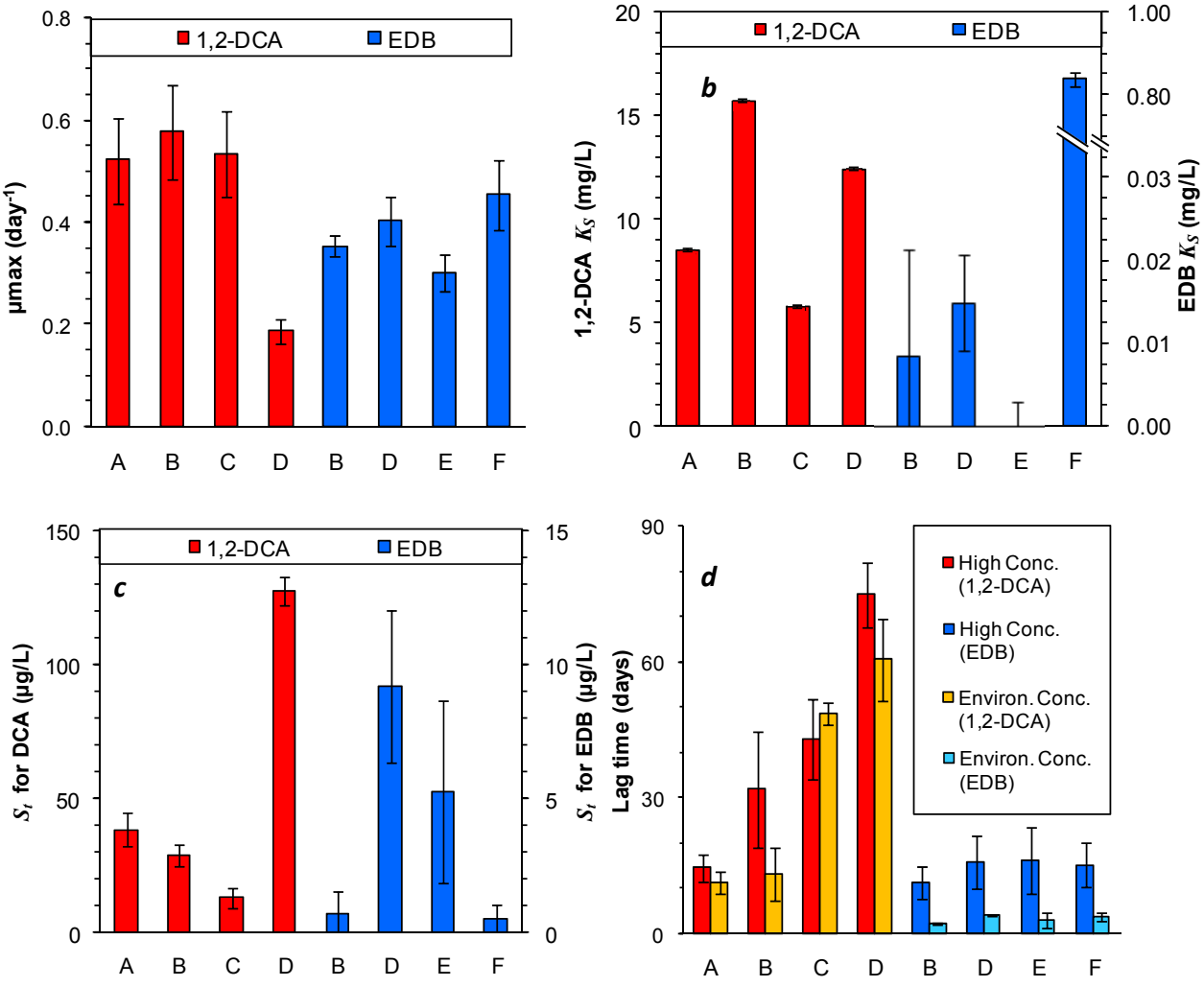


Figure 3.3 Summary of average μ (***a***), K_S (***b***), S_t values (***c***), and lag times (***d***) for 1,2-DCA and EDB in enrichment cultures grown on 1,2-DCA and EDB. Treatments are defined in Table 2.1. Error bars represent the standard deviation for 6-14 bottles per treatment.

.

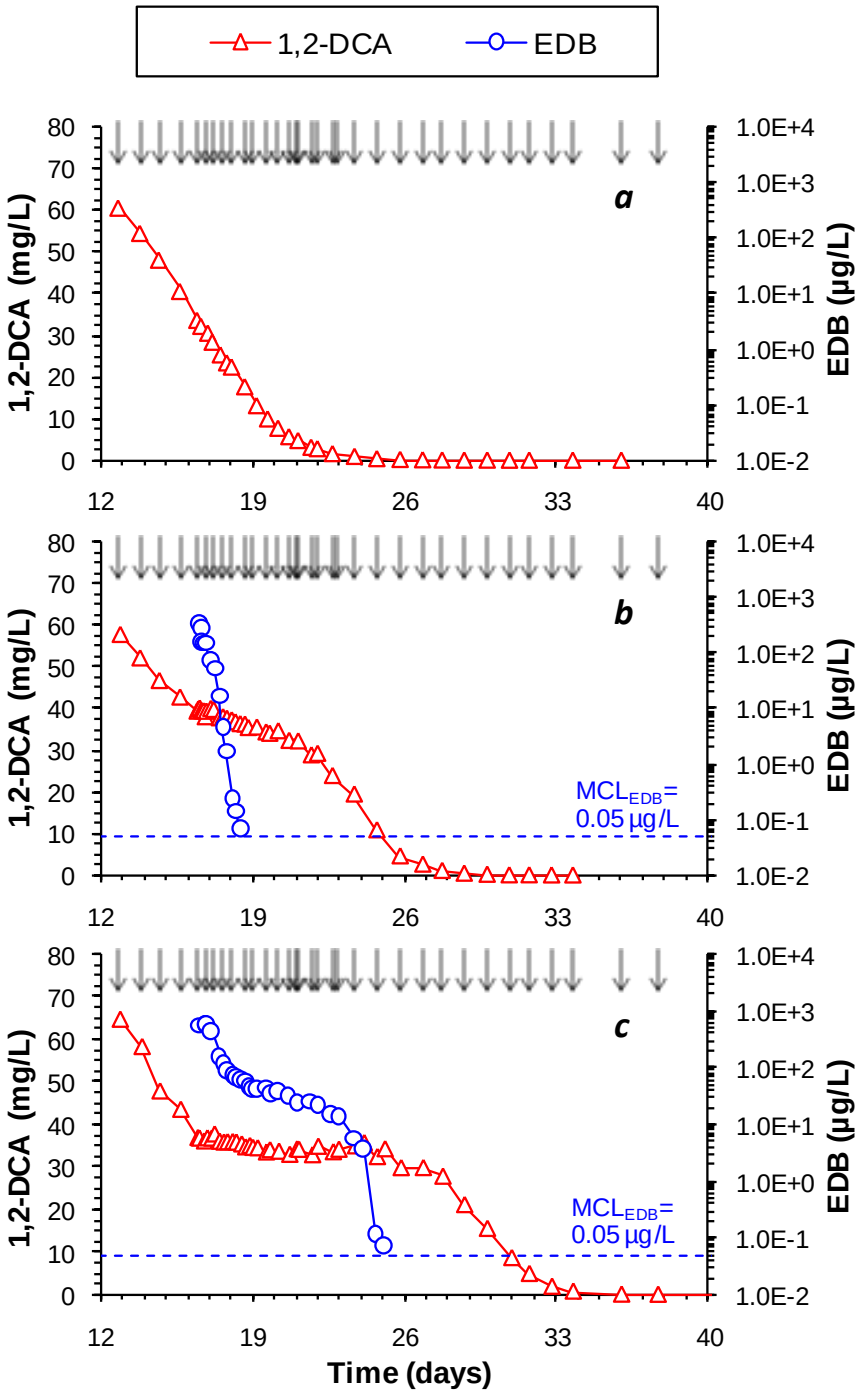


Figure 3.4 Representative results for the effect of EDB added to the enrichment culture biodegrading high levels of 1,2-DCA with no EDB added (***a***); 370 µg/L EDB added (***b***); and 645 µg/L EDB added (***c***). Each arrow indicates addition of 0.31 mmol lactate. Replicate bottles are shown in Supporting Information. EDB was added on day 16.5 and is shown in log scale (MCL = 0.05 µg/L).

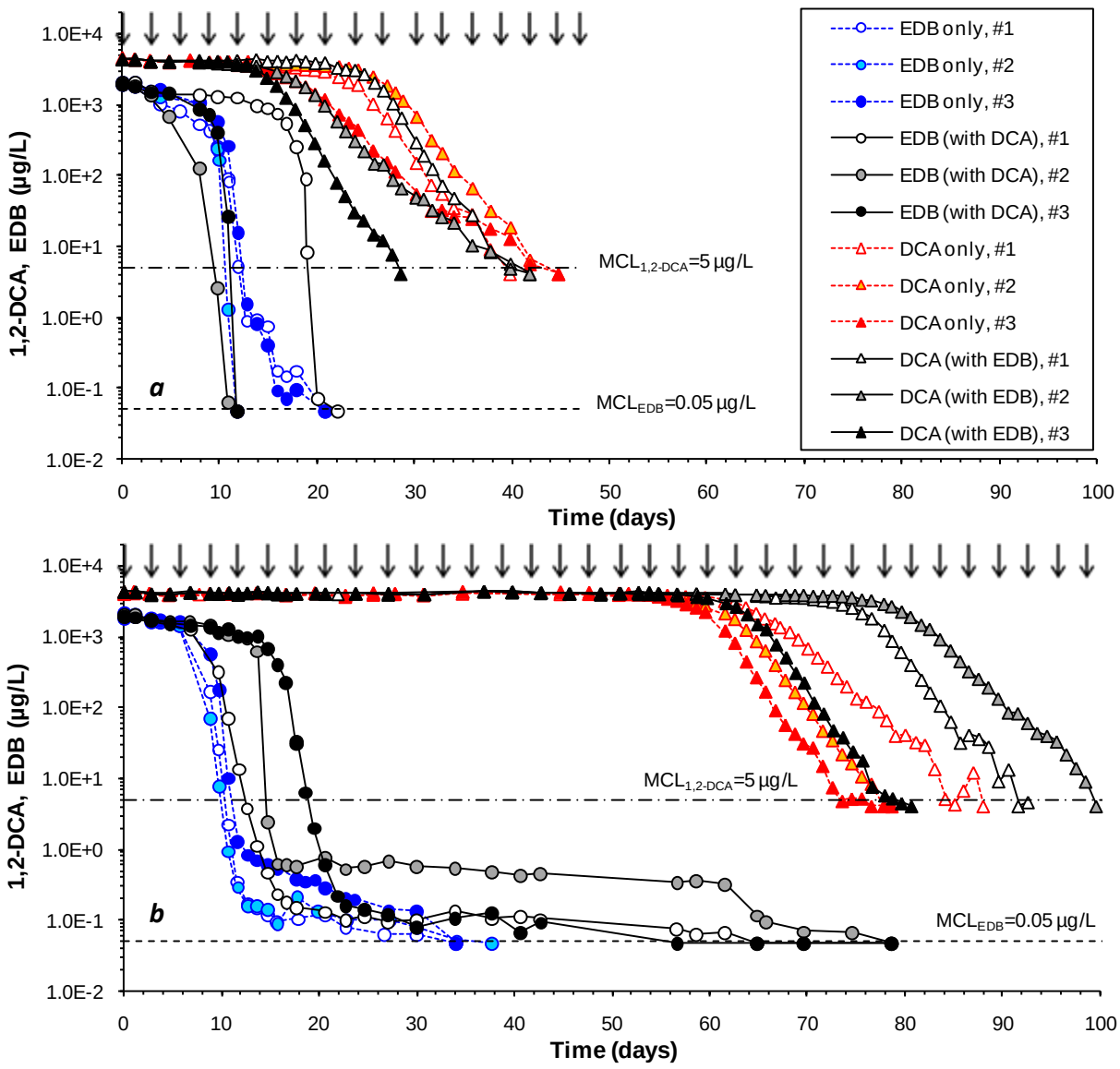


Figure 3.5 Biodegradation of environmental levels of 1,2-DCA or (and) EDB in an enrichment culture grown with 1,2-DCA as the terminal electron acceptor (***a***) and in an enrichment culture grown with EDB as the terminal electron acceptor (***b***). Each treatment was done in triplicates. Results for all the individual bottles are shown here. Each arrow indicates addition of 0.25 mmol lactate.

APPENDICES

Appendix-1: Pathway for 1,2-DCA and EDB Biodegradation Under Anaerobic Conditions

The pathway for anaerobic reductive dehalogenation of 1,2-DCA and EDB is mentioned in the Introduction. Figure A-1.1 shows the principal reductive dechlorination pathways for 1,2-DCA. The pathway for EDB is identical, with the substitution of bromine for chlorine.

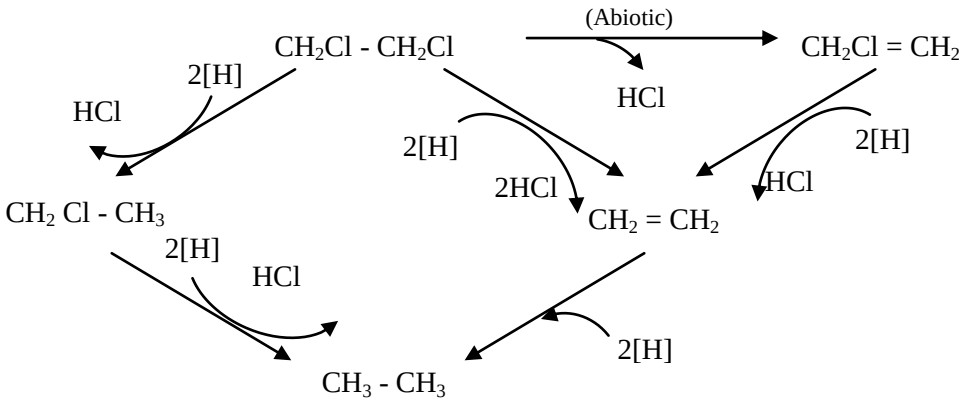


Figure A-1.1 Pathways for anaerobic reduction of 1,2-DCA; [H] = $H^+ + e^-$. The pathways for anaerobic transformation of EDB are identical.

Appendix-2: Enrichment Cultures

In the Materials and Methods section, under “Enrichment Cultures” mention is made of routine monitoring data and maintenance procedures for the cultures. The purpose of this appendixis to provide this information.

A-2.1 Maintenance of Enrichment Cultures

EDB and 1,2-DCA enrichment cultures were developed by in two 2.6 L glass reagent bottles. The bottles were placed in boxes to exclude light and incubated quiescently. Teflon-faced rubber septa (35 mm) were used to seal the bottles. The septa were placed inside a plastic bottle cap with 24-33 holes drilled for sampling by syringe. The bottles were incubated horizontally to maintain contact between the liquid and the septa, to minimize diffusive losses through the holes in the septa. The septa were replaced approximately once per month. The procedures used to maintain each enrichmentculture are described below.

An enrichment culture that chlororespires all of the chlorinated ethenes was used as the inoculum and 1,2-DCA or EDB were fed to the corresponding enrichment cultures. The 1,2-DCA enrichment culture was fed weekly by injecting 30 μL of neat 1,2-DCA; the actual amount added was determined gravimetrically. Each dose provided approximately 374 μmol per bottle and achieved an aqueous phase concentration of 0.24 mM (23.8 mg/L). The stoichiometric amount of electron donor required for dihaloelimination to ethene was 0.748 millielectron equivalents per bottle. To provide this plus a 75-fold excess (i.e., 55.7 millielectron equivalents per bottle), 1.9 mL of a sodium lactate stock solution (456.2 g/L of 60% sodium lactate syrup; 2.44 mol/L) was added.

The EDB enrichment culture was fed weekly with 10 mLof EDB-saturated water. The actual dose (approximately 204μmol per bottle) was determined by headspace analysis on the gas chromatograph. Initially, EDB was added as a neat liquid, in the same manner as 1,2-DCA. However, it tended to take so long for the EDB liquid to dissolve that the decision was made to switch to EDB-saturated water. The large volume needed (i.e., 10 mL) was inconvenient but it solved the problem with reaching equilibration. This amount of EDB added resulted inapproximately the same aqueous phase concentration as 1,2-DCA, i.e., 0.13 mM(24.5 mg/L). Due to differences in molecular weights and Henry's law constants, achieving the same aqueous phase concentratin resulted in adding a different total amount of EDB, i.e., 204 μmol per bottle of EDB per dose versus 374 μmol per bottle per dose of 1,2-DCA. Thestoichiometric amount of electron donor required for dihaloelimination of each dose of EDB to ethene was 0.408 millielectron equivalents per bottle.A dose of 0.67 mL per bottle of lactate was added to the EDB enrichment culture, providing 19.6 millielectron equivalents, or a 50-fold excess.

After four additions of 1,2-DCA were consumed, 150 mL of the mixed culture was removed and replaced with MSM. After three additions of EDB, 200 mL of the mixed culture was removed and replaced with MSM. This action prevented the build-up of headspace gas pressure (from ethene and methane) and the accumulation of salts (from neutralization of HCl and HBr with NaOH) in the liquid. Every time a certain amount of enrichment culture was used for inoculation to start an experiment, the same volume of MSM was added in compensation.

A-2.2 Monitoring Data for the Enrichment Cultures

The performance of the 1,2-DCA enrichment culture over the period of time when it was used during this study is shown in Figure A-2.1. The predominantdehalogenation product was

ethene; minor amount of ethane and VC also accumulated. The percent recovery of daughter products is provided in section5.0.

The performance of the EDB enrichment culture over the period of time when it was used during this study is shown in Figure A-2.2. The predominant dehalogenation product was ethene; minor amount of ethane and VC also accumulated. The percent recovery of daughter products is provided inAppendix section 5.

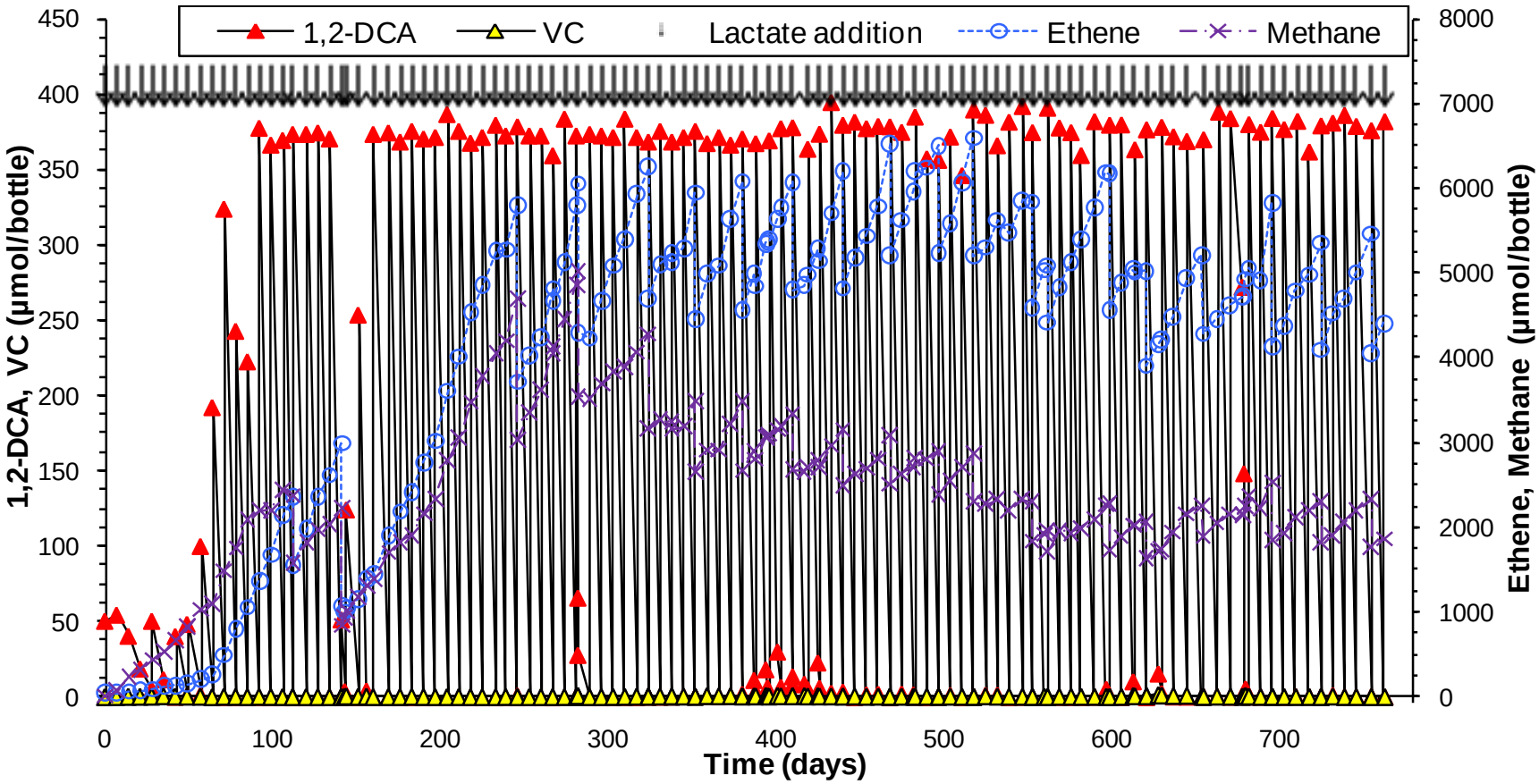


Figure A-2.1 Data for 1,2-DCA enrichment culture maintenance.

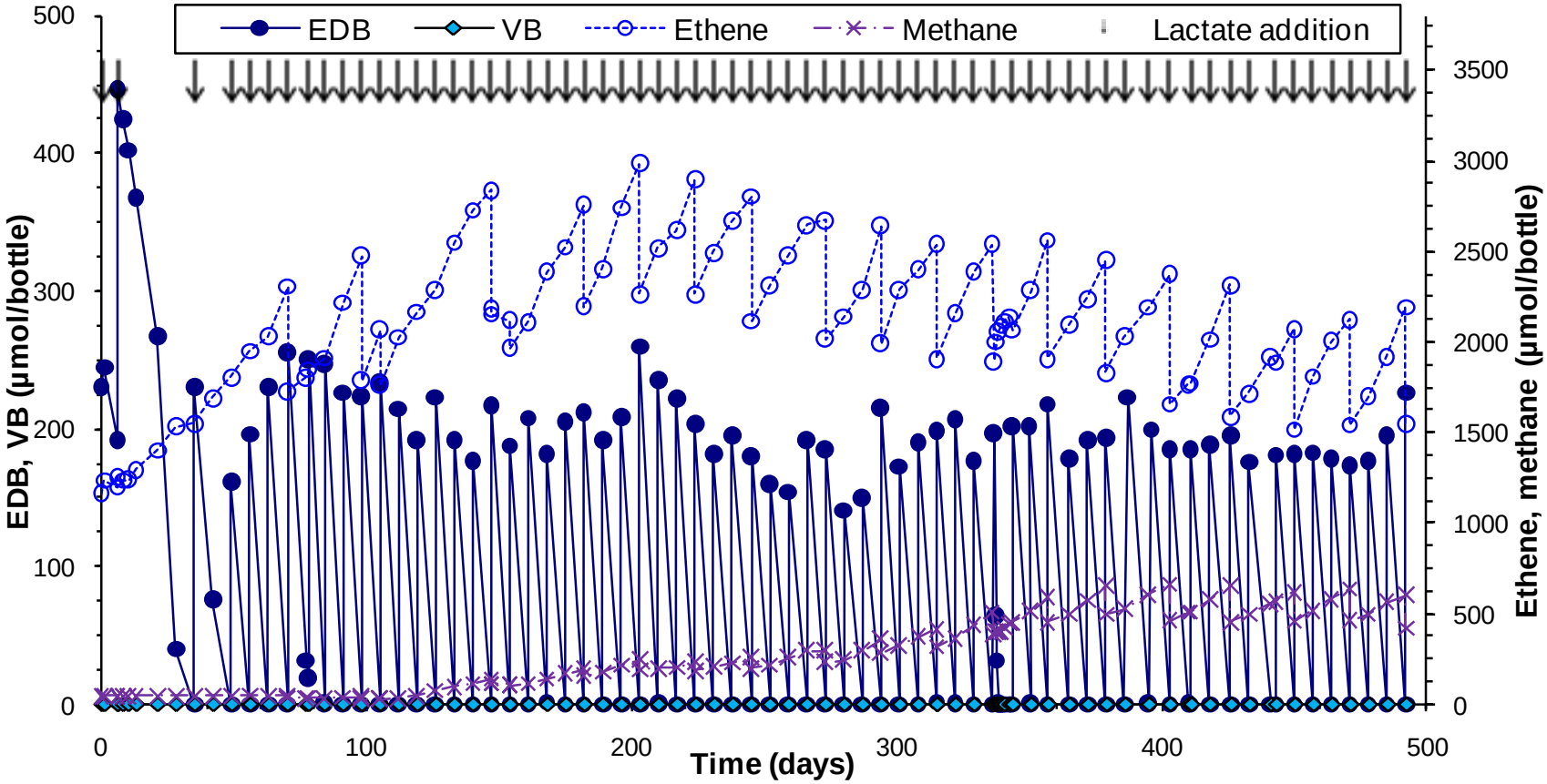


Figure A-2.2 Data for EDB enrichment culture maintenance.

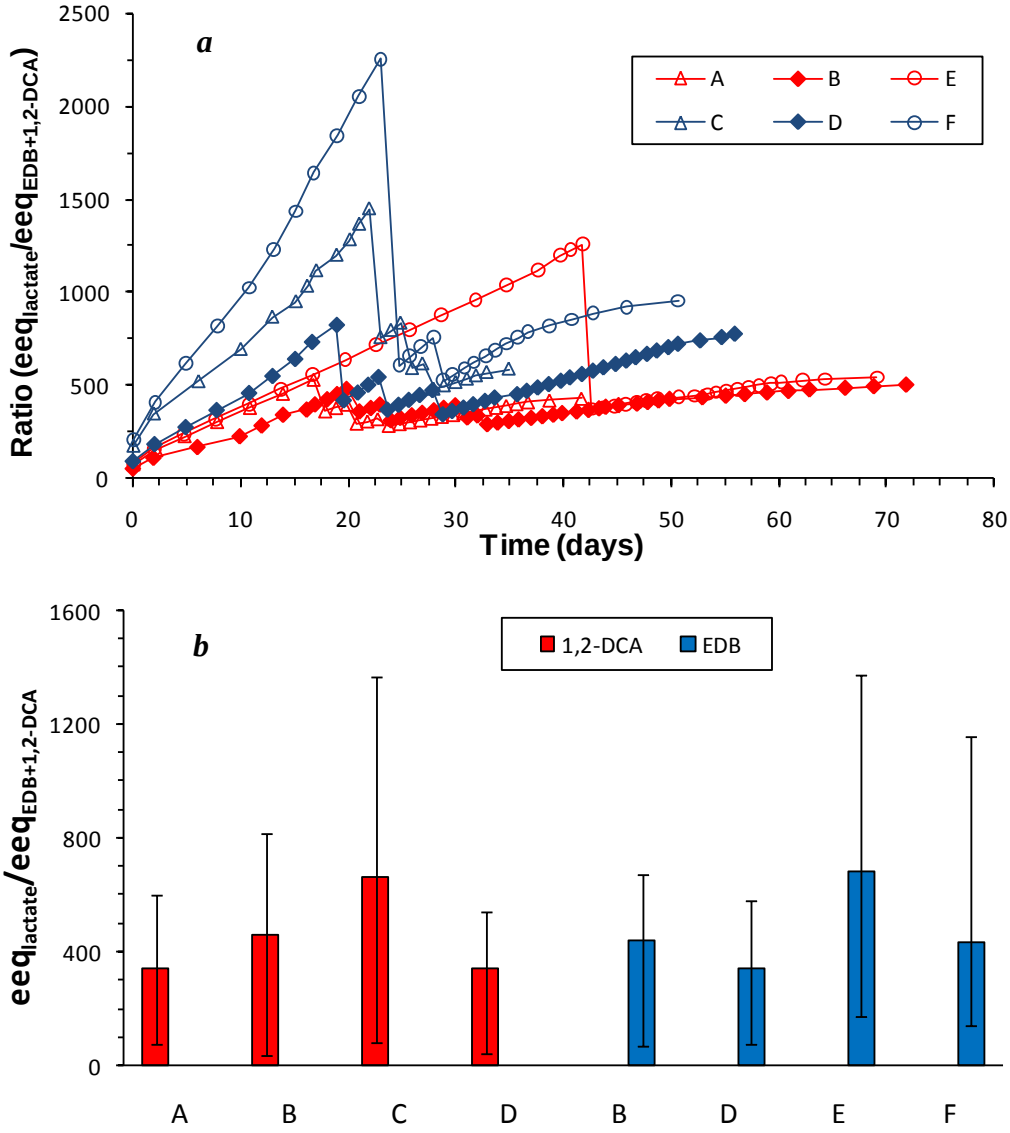
Appendix-3: Magnitude of Electron Donor Additions

In the Materials and Methods section, under "Batch Kinetics at High Concentrations", mention is made of providing lactate in considerable excess of the amount needed for stoichiometric reduction of 1,2-DCA and EDB. It was also stated that acetate and propionate were the major organic acids that accumulated. The purpose of this section of the Appendix is to provide documentation of this assessment.

Figure A-3.1a shows the ratio of the cumulativeelectron donor equivalents (eeq) of lactate added to the cumulative electron acceptor equivalents needed for stoichiometric reduction of 1,2-DCA and EDB. Each curve represents a single bottle from each treatment; results for the other bottles were similar. The ratio ranged from 15 at the start of the incubation to as high as 177.Figure A-3.1b provides a summary of average ratio based on results from all the bottles in each treatment over the whole incubation period, as well as the minimum and the maximum ratio.

Figure A-3.2 shows the disposition of lactate that was added to four bottles: two from treatment A (inoculated with the 1,2-DCA enrichment culture and supplied with 1,2-DCA as the electron acceptor) and two from treatment F (inoculated with the EDB enrichment culture and supplied with 1,2-DCA as the electron acceptor), over the first 6.5 days of incubation. The cumulative amount of lactate added is shown as a step function; this was calculated based on the volume and concentration of the lactate stock solution. Also shown are the amounts of acetate, propionate and remaining lactate, based on HPLC measurements of filtered (0.45 μm) samples. As expected, there was a significant level of lactate consumption (based on the difference between the amount added and the measured amount remaining). By the end of the monitoring

period, the sum of the concentration of the three tested organic acids was close to the cumulative lactate concentration added (i.e., 22-24 mM), indicating a stoichiometric fermentation of lactate to acetate and propionate.



A: 1,2-DCA in a culture grown with 1,2-DCA as TEA

B: 1,2-DCA + EDB in a culture grown with 1,2-DCA as TEA

C: 1,2-DCA in a culture grown with EDB as TEA

D: 1,2-DCA + EDB in a culture grown with EDB as TEA

E: EDB in a culture grown with 1,2-DCA as TEA

F: EDB in a culture grown with EDB as TEA

Figure A-3.1 Ratio of electron equivalents of lactate added to electron equivalents needed for complete dehalogenation over time (***a***); and the average electron equivalents of lactate added to electron equivalents needed for complete dehalogenation (***b***). The bars in panel b show the maximum and minimum ratios.

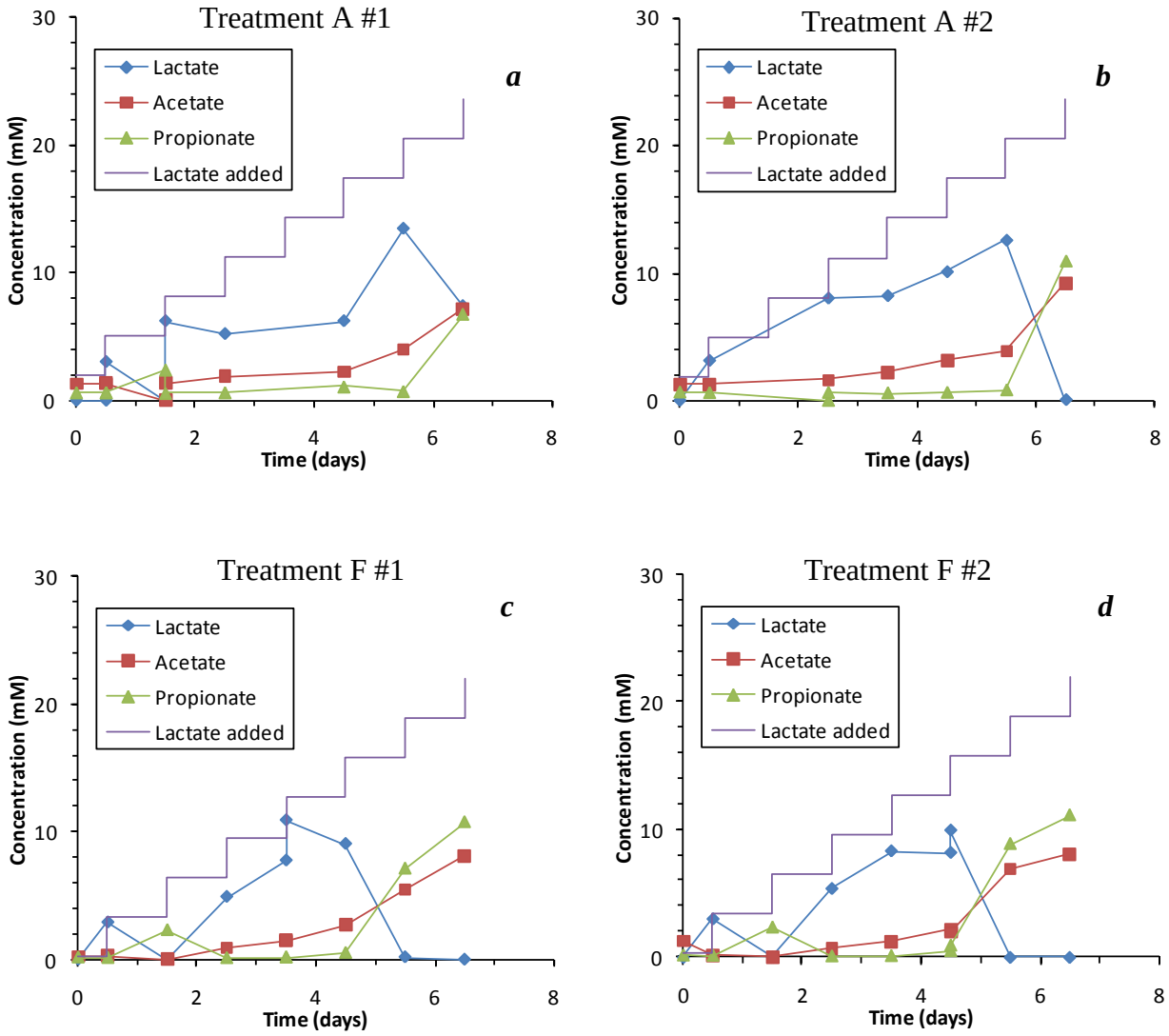


Figure A-3.2 Lactate additions and organic acid concentrations in four bottles; **(*a*) treatment A, bottle #1; (*b*) treatment A, bottle #2; (*c*)** treatment **F, bottle #1; and (*d*)** treatment F, bottle #2.

Appendix-4: Evaluation of Strategies for Adding 1,2-DCA and EDB

In the Materials and Methods section, under "Batch Kinetics at High Concentrations", mention is made of the strategy used to add 1,2-DCA and EDB for the high concentration kinetic tests. The selection of anaddition method was based onseveral preliminary tests, which are covered in this section of the Appendix. A variety of methods were explored.Resultsforfour approachesareshown in Figure A-4.1 for 1,2-DCA and Figure A-4.2 for EDB. Monitoring data for two representative bottles are shown in each figure.

One of the methods tested with 1,2-DCA was an initially moderate dose, followed by repeated additions to 40-50 µmol/bottle over the incubation period (Fig. A-4.1, panels a and b). A second approach was to add a lower initial dose followed by gradually increasing doses (Fig. A/4.1c) or a single high dose (Fig. A-4.1d), so that the highest amount added wasabove 500 µmol/bottle. A third approach evaluated was to provide a highinitial dose (90-110 µmol/bottle) and no others (Fig. A-4.1, panels e and f). The fourth strategy was to provide a low initial dose, followed by repeated additions at increasing doses (Fig. A-4.1, panels g and h). This strategy was selected since it did not cause inhibition at the start (due to high initial concentrations), yet allowed for subsequent addition of high enough concentrations to capture the maximum growth rate and half saturation coefficient.

Figure A-4.2 shows of the same strategies for EDB addition, although the initial amounts and highest amounts added were generally lower than for 1,2-DCA. As with 1,2-DCA, the fourth approach (i.e., a low initial dose, followed by repeated additions at increasing doses) was adopted since it avoided problems caused by inhibition with high initial concentrations, yet allowed for quantification of the maximum growth rate and half saturation coefficient.

Method (d) in Figure A-4.2 proved to be suitable. This is analogous to the addition pattern selected for 1,2-DCA, with one exception that the highest EDB level present in the bottle was not allowed to exceed 50 μmol/bottle due to an observed inhibitory effect. 1,2-DCA can be increased to more than 100 μmol/bottle without an inhibitory effect.

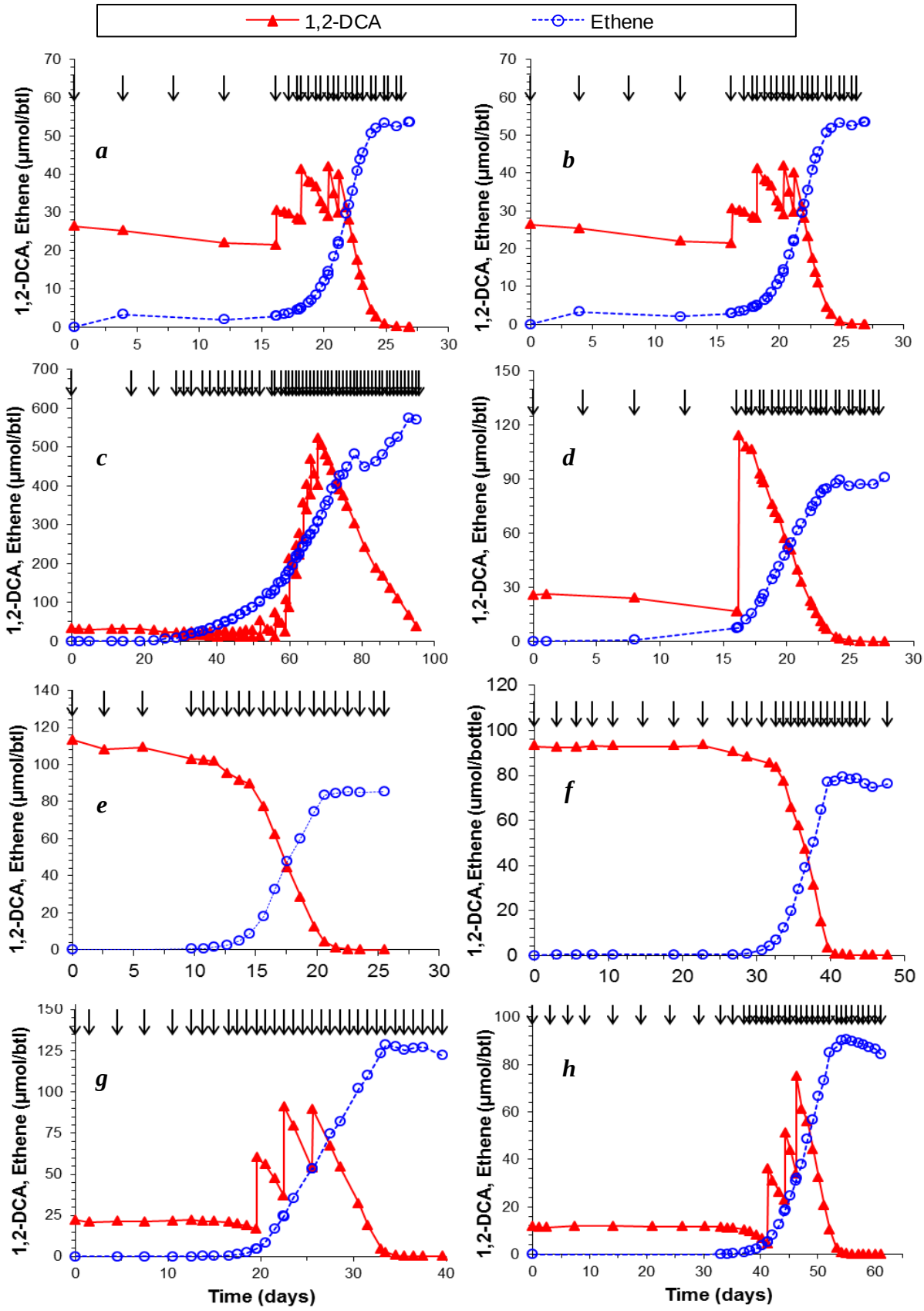


Figure A-4.1 Comparison of different patterns of 1,2-DCA addition; see the text for a description of the feeding strategy for each panel. Arrows indicate lactate additions.

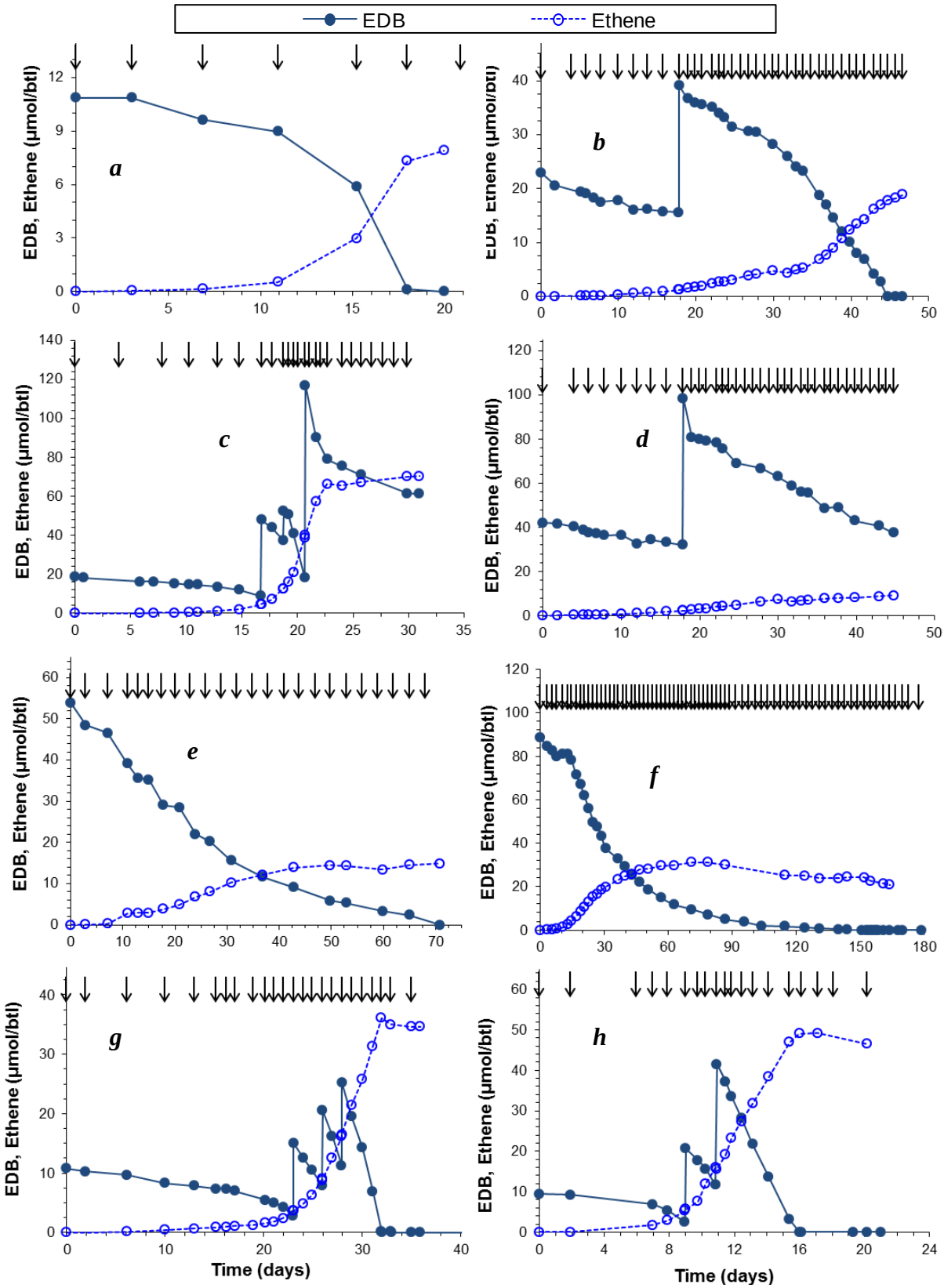


Figure A-4.2 Comparison of different patterns of EDB; see the text for a description of the feeding strategy for each panel . Arrows indicate lactate additions.

Appendix-5: Percent Recoveries of Ethene, VC, VB and Methane

In the Results section, under “High Concentrations of 1,2-DCA and EDB”, mention is made of the percent recoveries of daughterproducts from 1,2-DCA and EDB dehalogenation, as well as the amount of methane formed. This section of the Appendixprovides the supporting information for theseassessments.

FigureA-5.1a shows the percent recovery of ethene. It was calculated by dividing the total amount of ethene formed by the total amount of 1,2-DCA and EDB consumed, on a molar basis. Recoveries ranged from 65.1-77.6%. FigureA-5.1b shows the maximum amount of VC and VB that accumulated in each treatment. The percentages were calculated based on the highest amount of VC or VB that accumulated over the entire incubation period, divided by the total amount of 1,2-DCA and EDB consumed, respectively, on a molar basis. The percentages ranged from 0.40% to 0.68% for VC, and 0.052% to 0.093% for VB. Figure A-5.1c shows the amount of methane formed. These values were calculated based of the electron equivalents of methane formed (moles of methane * 8 eeq/mole) divided by the electron equivalents of lactate added (moles of lactate * 12 eeq/mole), during the time periods when 1,2-DCA or EDB were being consumed. All of the treatments are less than or equal to 1%, indicating very significant inhibition of methanogenesis.

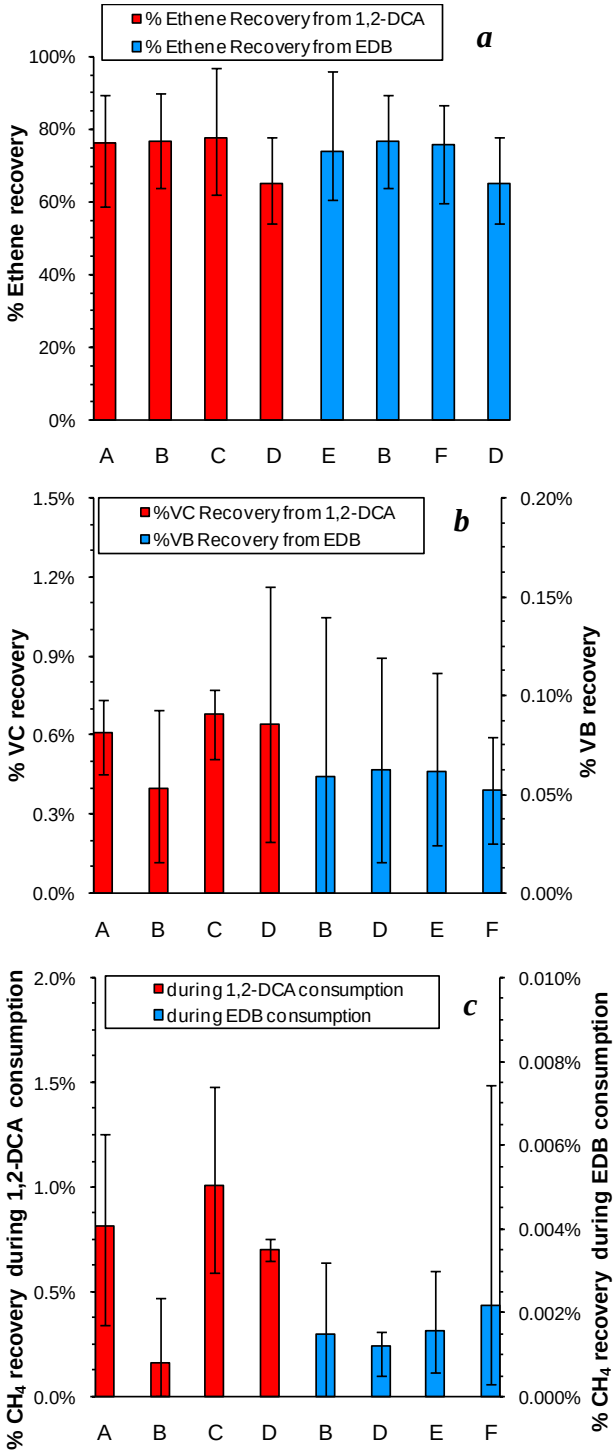


Figure A-5.1 Percent recoveries for (***a***) ethene; (***b***) VC, VB and (***c***) methane. Error bars represent the standard deviations based on all bottles in each treatment.

Appendix-6: Comprehensive Results for theHigh Concentration Testswith1,2-DCA and EDB

In the Results section, under “High Concentrations of 1,2-DCA and EDB”, results are presented for representative bottles in treatments A, B, D and F (Figure 3.1). The purpose of this section of the Appendixis to present the results for all of the bottles that were used in the kinetic analyses.

Results for treatment A are shown in Figures A-6.1, A-6.2, and A-6.3.

Results for treatment B are shown in Figure A-6.4.

Results for treatment C are shown in Figures A-6.5 and A-6.6.

Results for treatment D are shown in Figure A-6.7.

Results for treatment E are shown in Figure A-6.8 and A-6.9

Results for treatment F are shown in Figures A-6.10 and A-6.11.

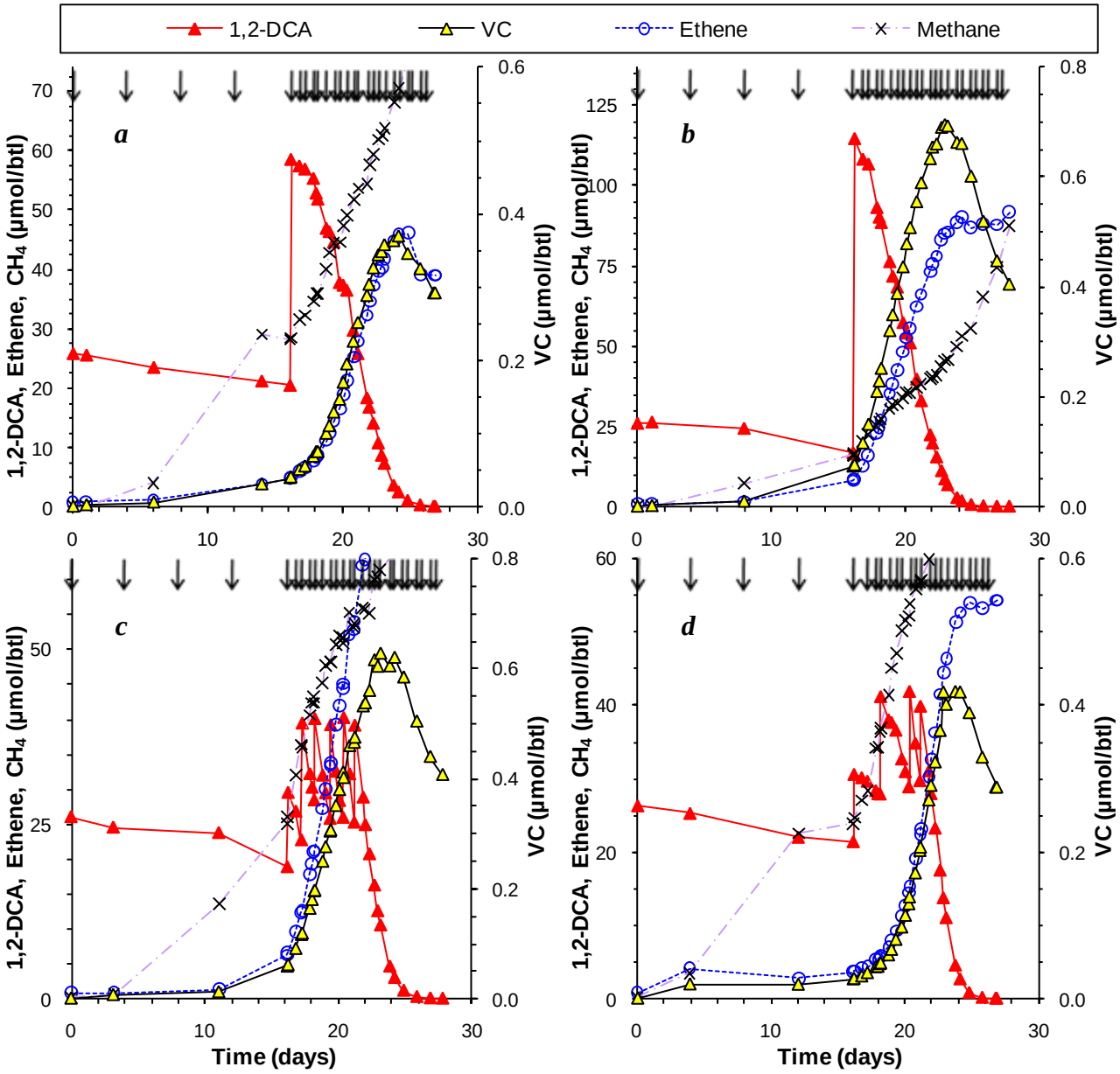


Figure A-6.1 Results for treatment A, (***a***) bottle #1; (***b***) bottle #2; (***c***) bottle #3; and (***d***) bottle #4.

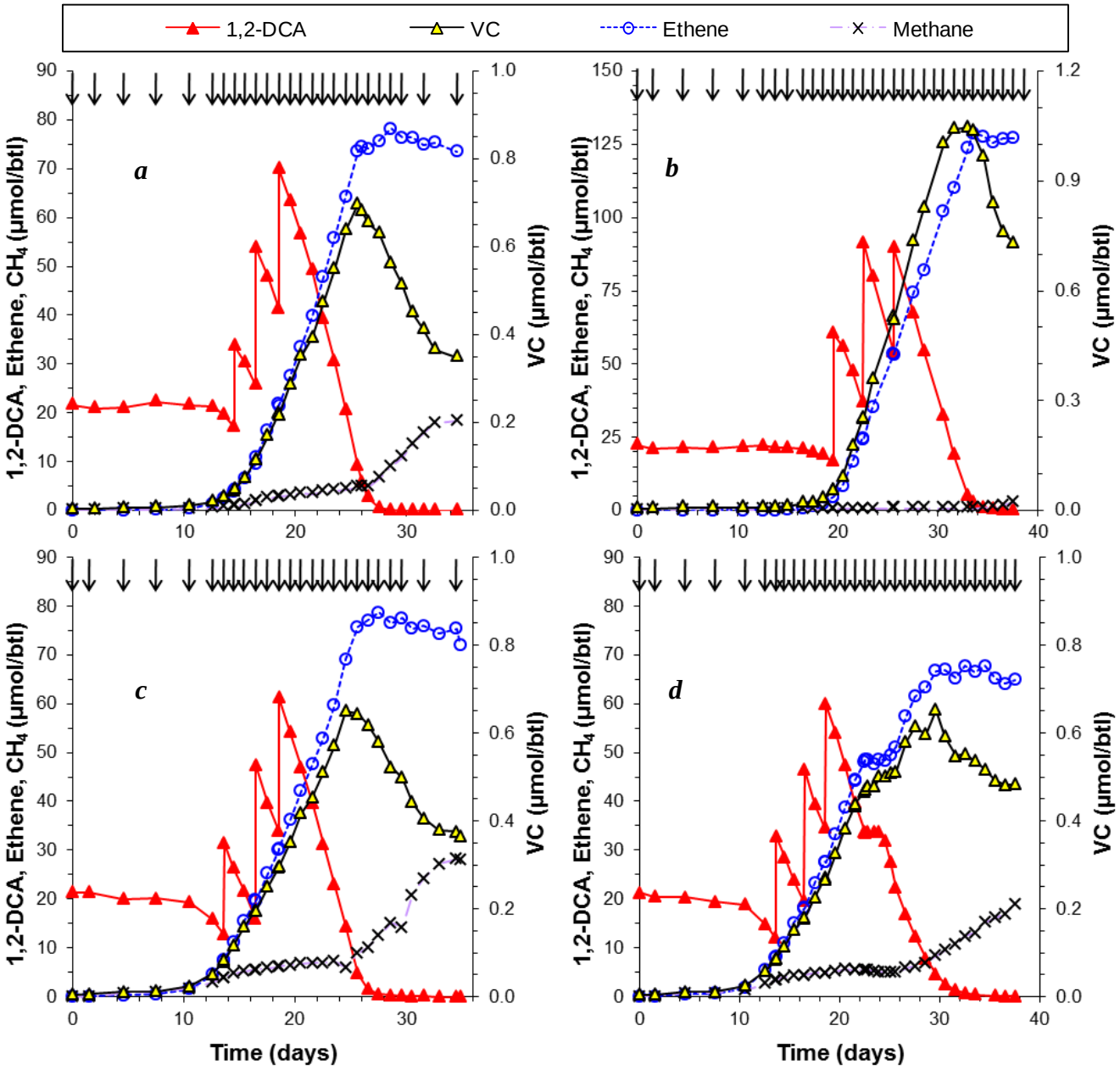


Figure A-6.2 Results for treatment A, (***a***) bottle #73; (***b***) bottle #74; (***c***) bottle #75; and (***d***) bottle #76.

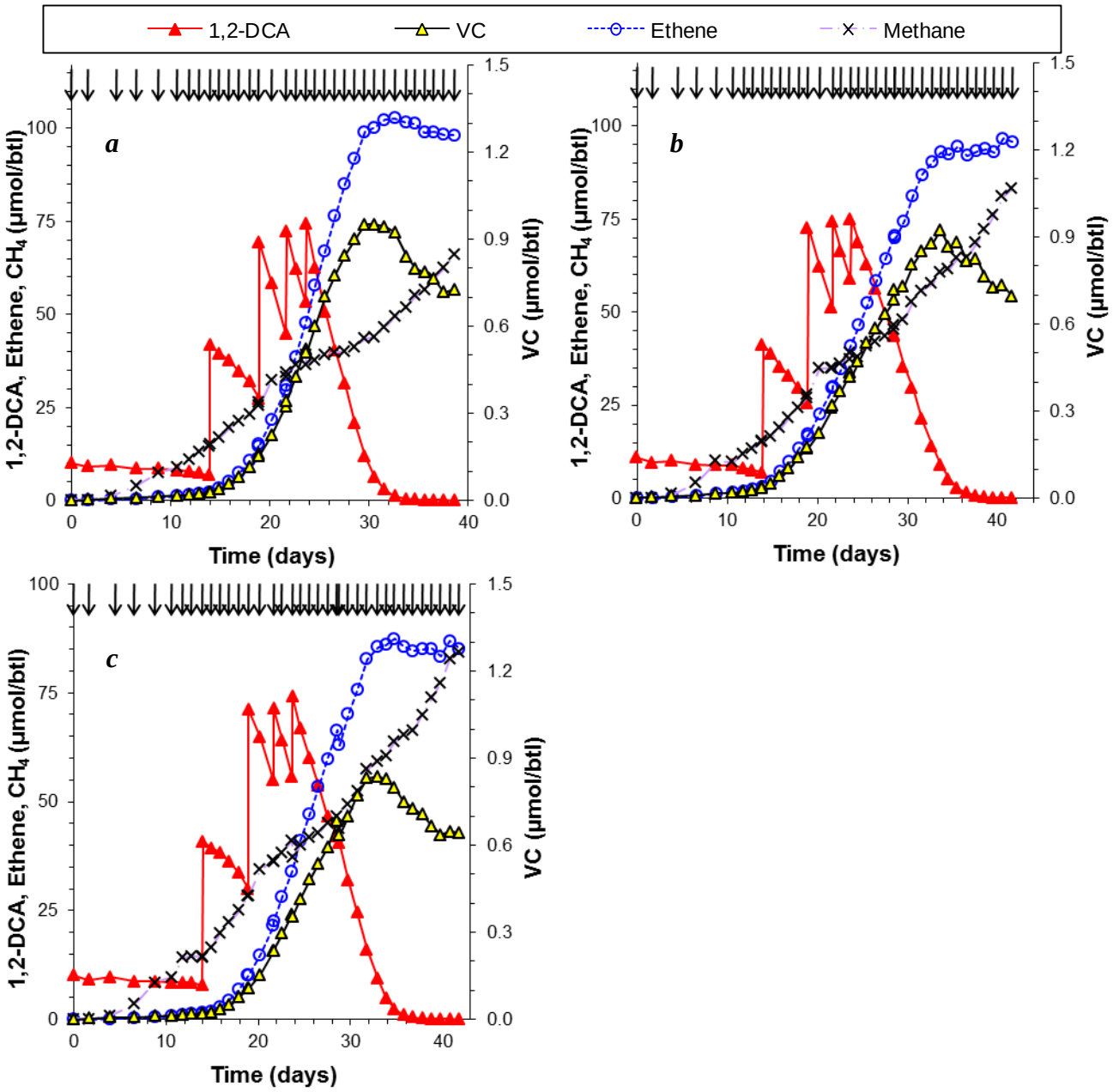


Figure A-6.3 Results for treatment A, (***a***) bottle #A1; (***b***) bottle #A2; and (***c***) bottle #A3.

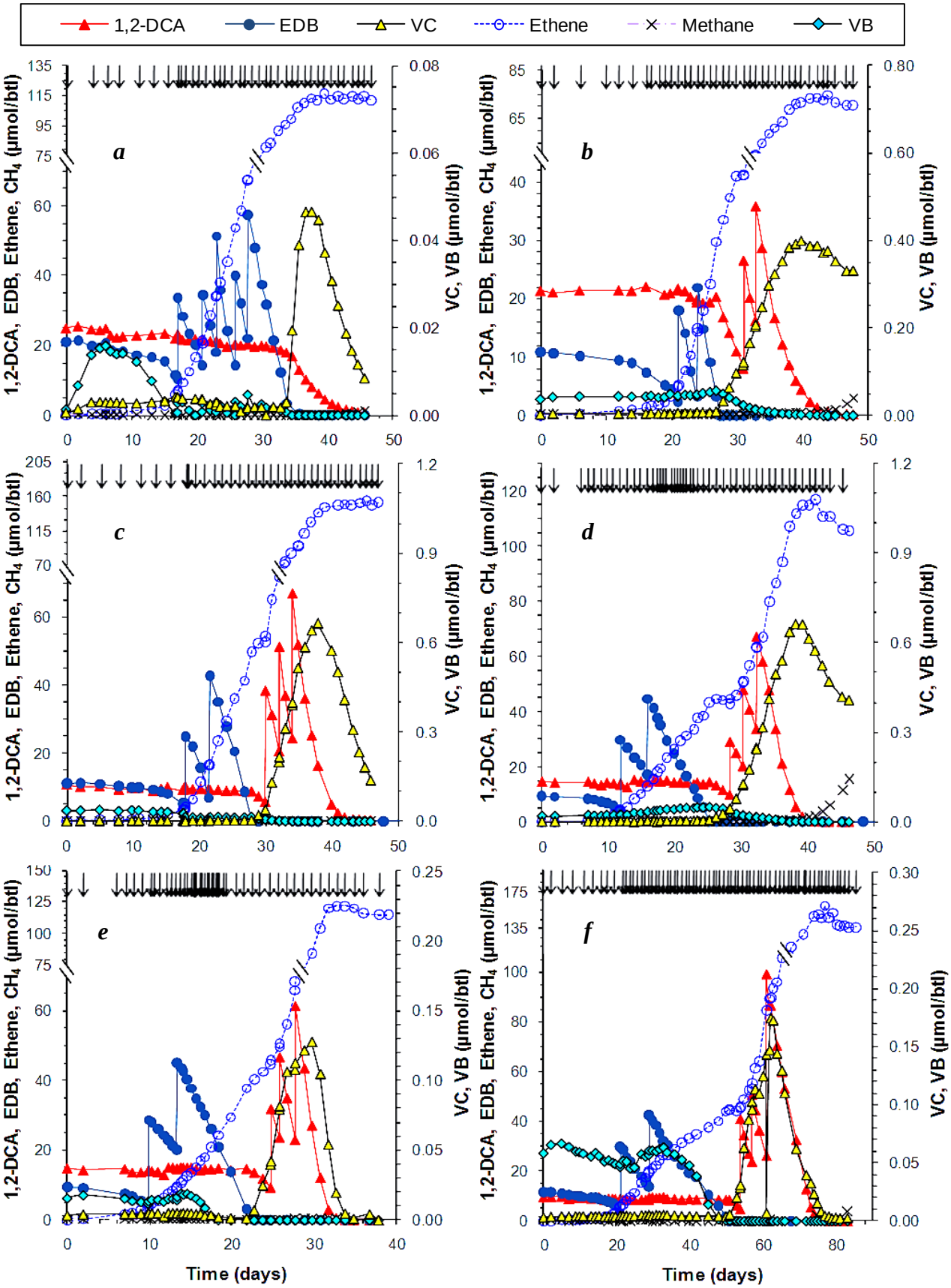


Figure A-6.4 Results for treatment B, (***a***) bottle #24; (***b***) bottle #90; (***c***) bottle #100; (***d***) bottle #109; (***e***) bottle #110; and (***f***) bottle #99.

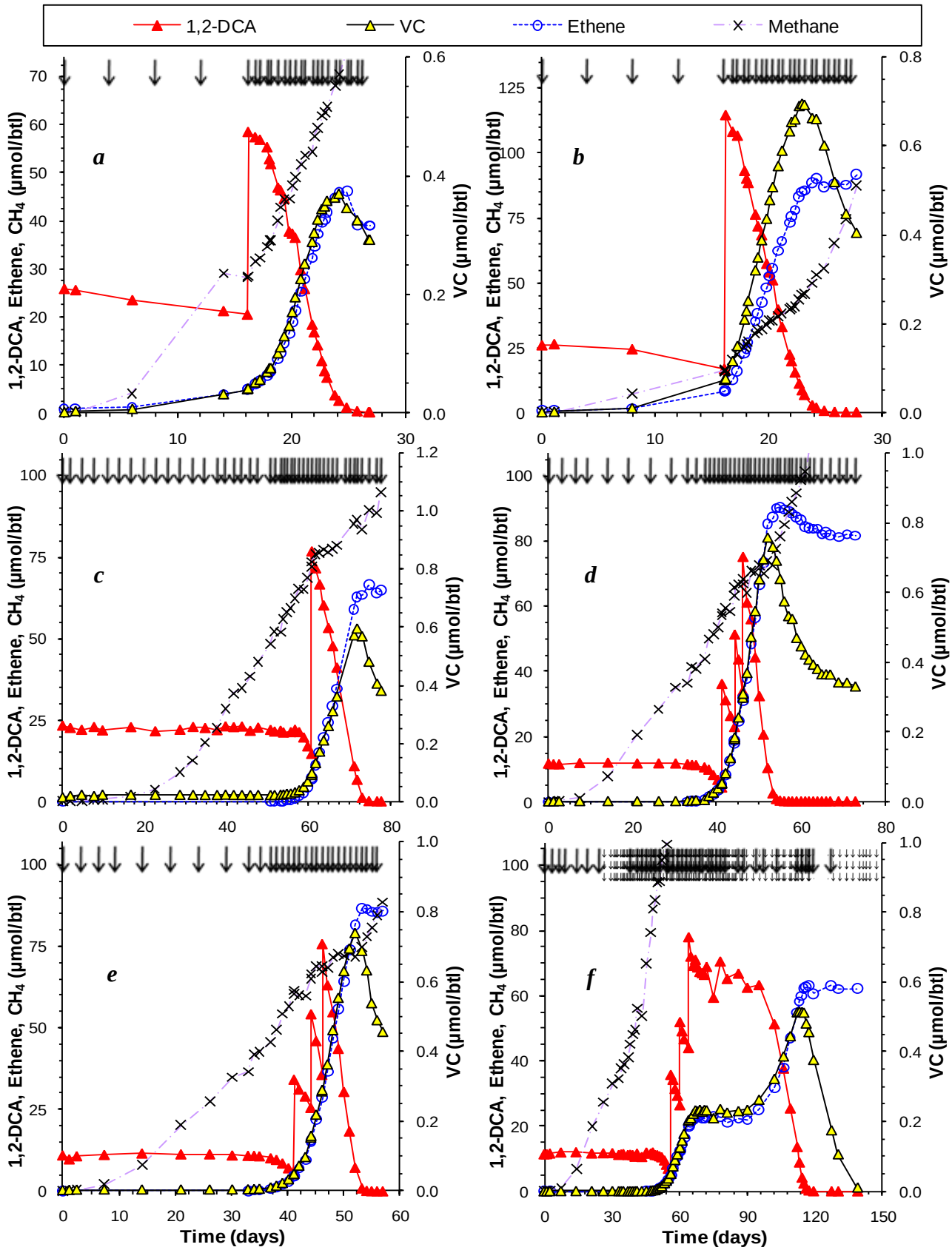


Figure A-6.5 Results for treatment C, (***a***) bottle #85; (***b***) bottle #86; (***c***) bottle #87; (***d***) bottle #102; (***e***) bottle #103; and (***f***) bottle #104.

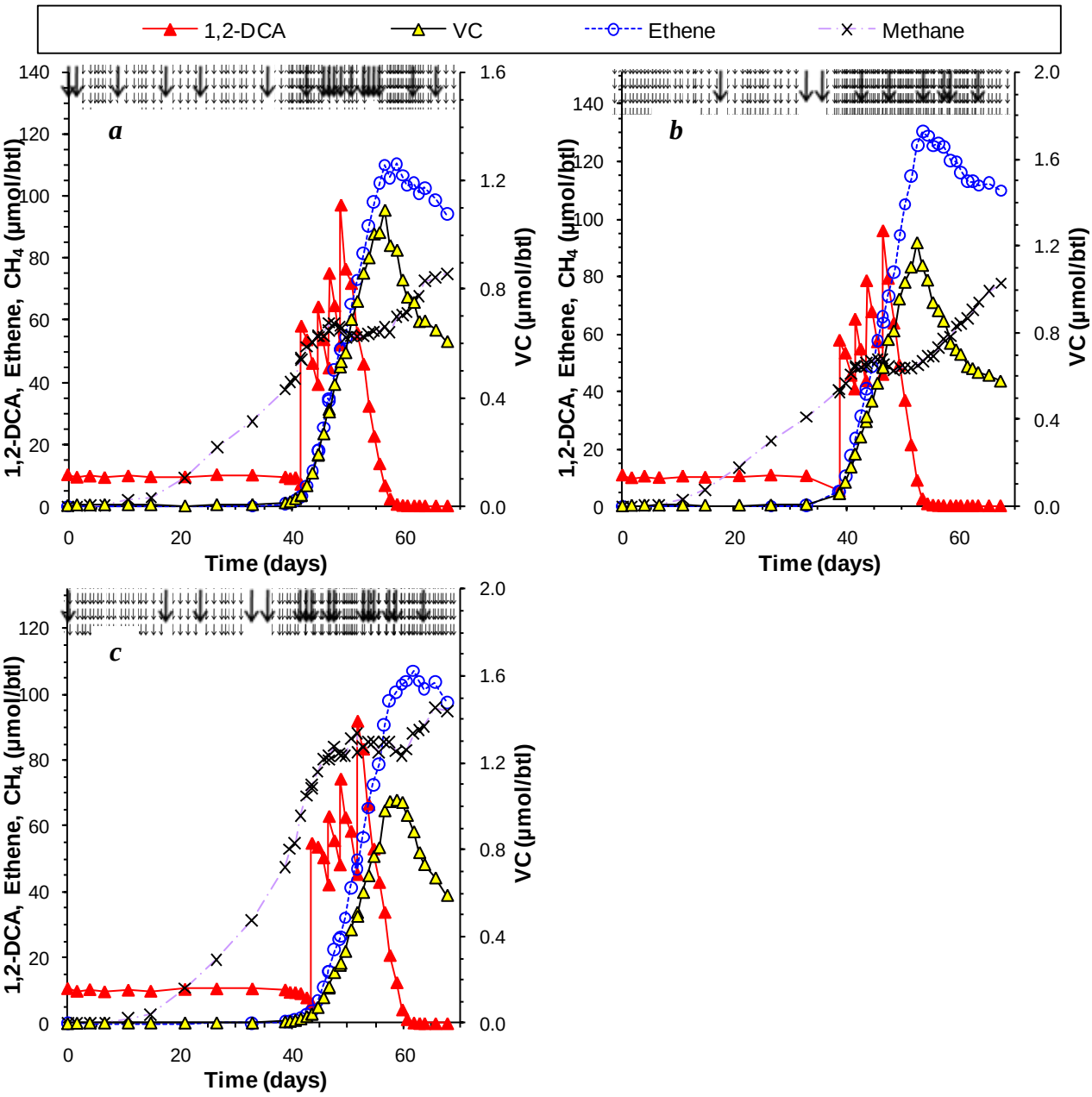


Figure A-6.6 Results for treatment C, (***a***) bottle #C1; (***b***) bottle #C2; and (***c***) bottle #C3.

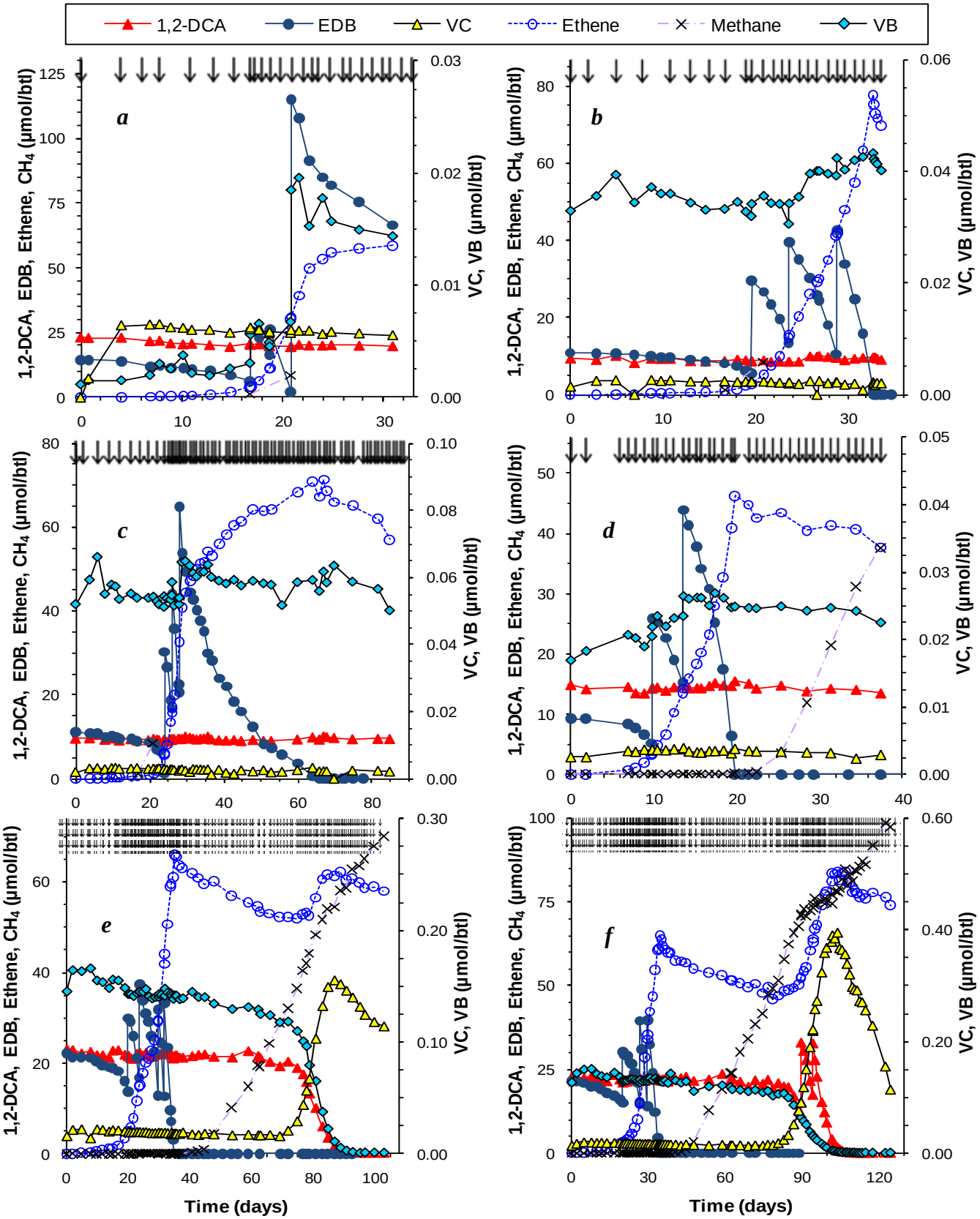


Figure A-6.7 Results for treatment D, (***a***) bottle #60; (***b***) bottle #105; (***c***) bottle #106; (***d***) bottle #113; (***e***) bottle #93; and (***f***) bottle #94.

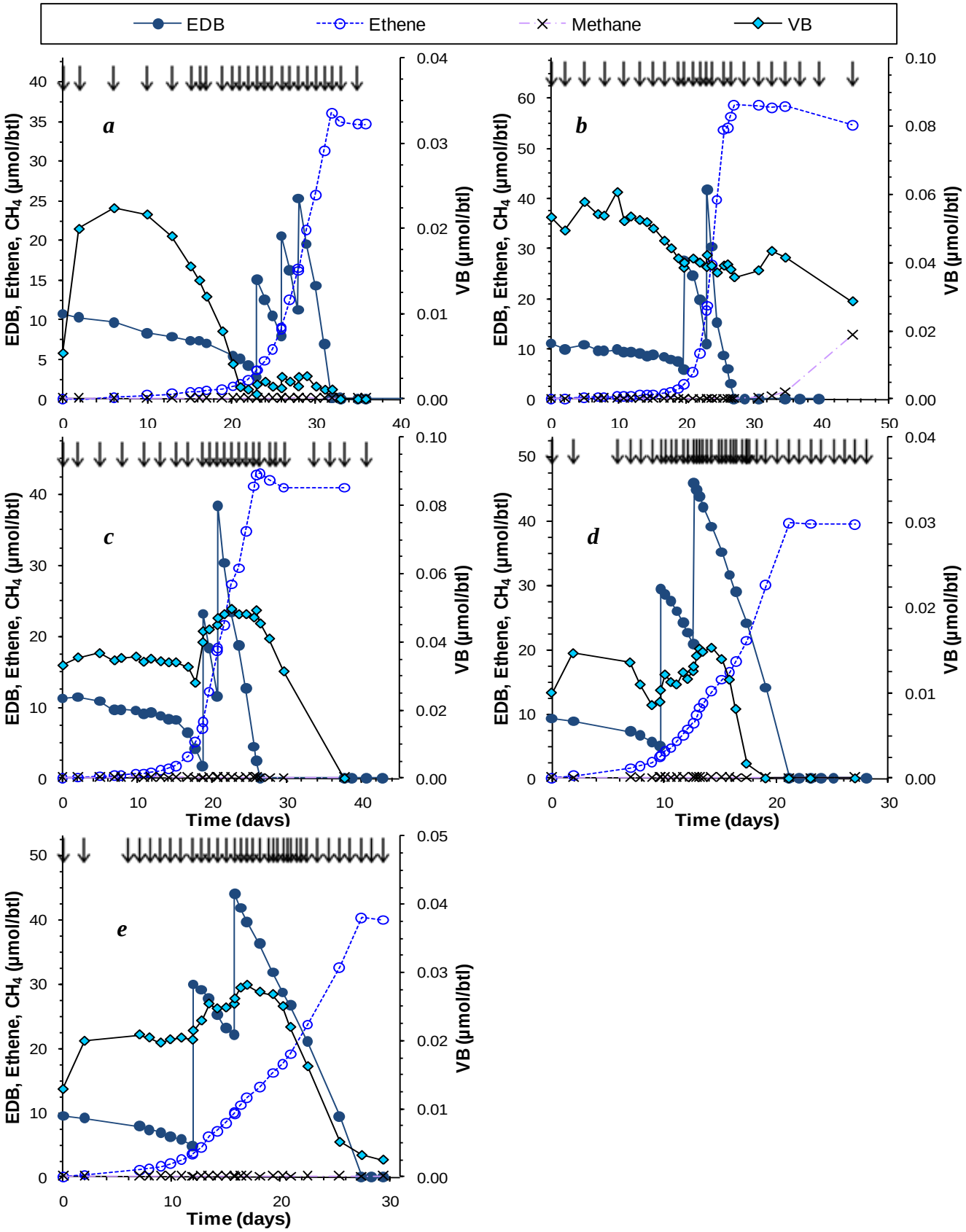


Figure A-6.8 Results for treatment E, (***a***) bottle #60; (***b***) bottle #105; (***c***) bottle #106; (***d***) bottle #113; (***e***) bottle #93; and (***f***) bottle #94.

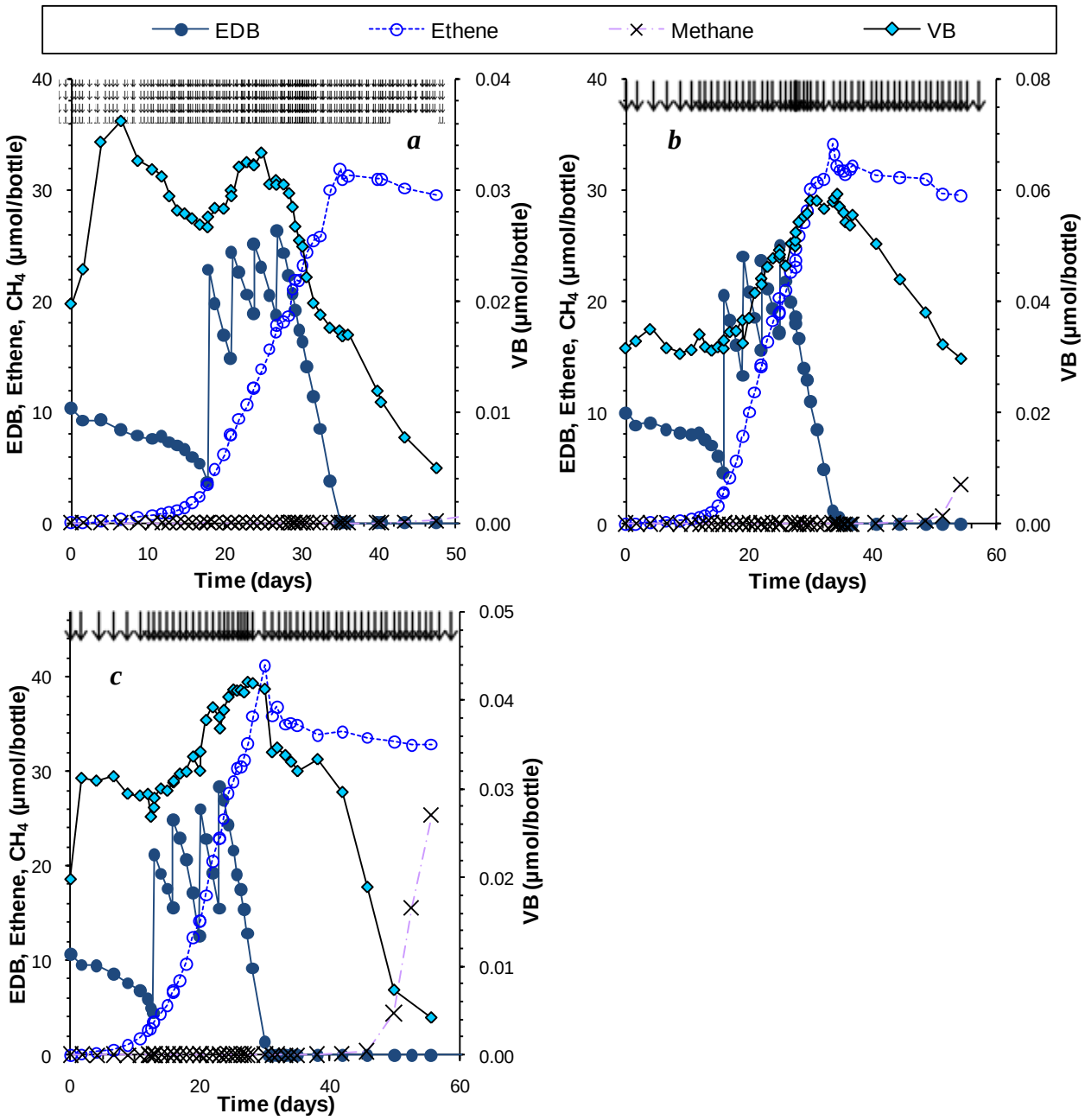


Figure A-6.9 Results for treatment E, (***a***) bottle E1; (***b***) bottle #E2; (***c***) bottle #E3.

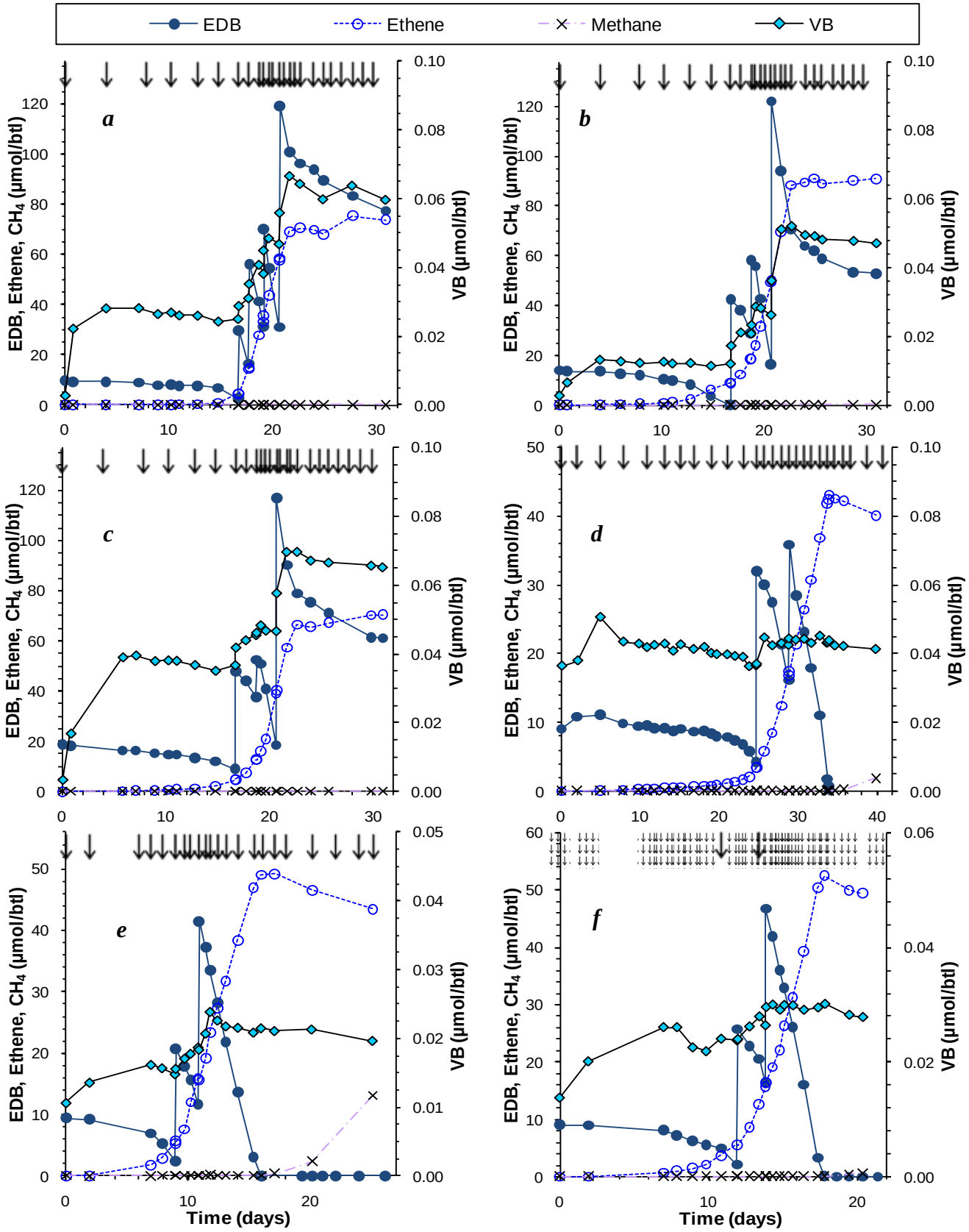


Figure A-6.10 Results for treatment F, (***a***) bottle #42; (***b***) bottle #43; (***c***) bottle #44; (***d***) bottle #101; (***e***) bottle #111; and (***f***) bottle #112.

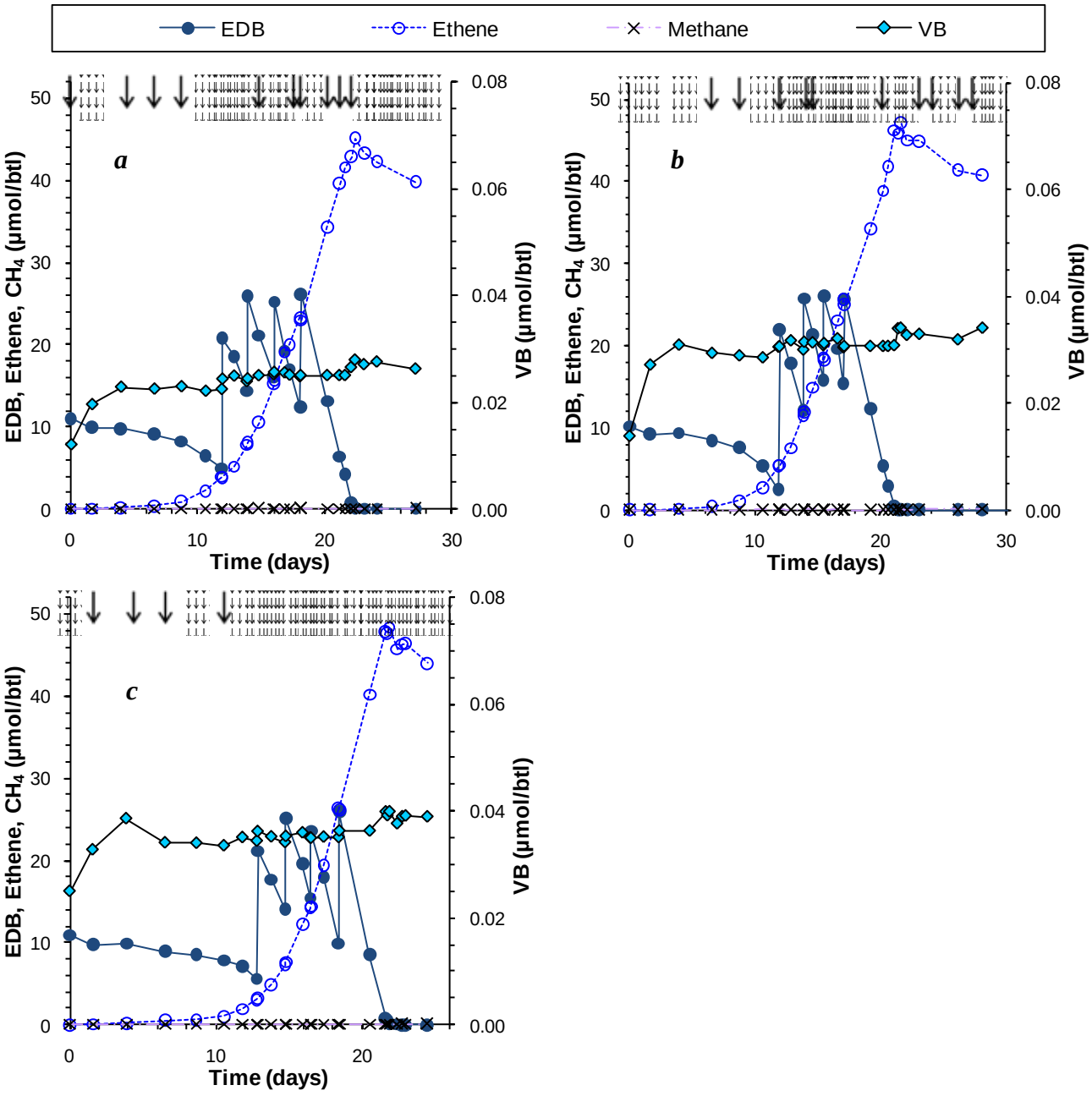


Figure A-6.11 Results for treatment F, (***a***) bottle #F1; (***b***) bottle #F2; and (***c***) bottle #F3.

Appendix-7: Mineral Medium Controls and Water Controls

In the Results section, under "High Concentrations of 1,2-DCA and EDB", mention is made of the results for mineral medium controls and water controls. The purpose of this section of the Appendixis to present these results.

Figure A-7.1 shows the results for the medium controls. Different amounts of 1,2-DCA and EDB were evaluated. One set of controls contained 1,2-DCA and EDB at levels close to those at gasoline spill sites, which were used in the low concentration experiments (i.e., ~4 µmol/bottle of 1,2-DCA, or 4 mg/L; ~1 µmol/bottle of EDB, or 2 mg/L). There was no substantial decrease in 1,2-DCA during 124 days of incubation, while there was a modest loss of EDB after day 47-55. At an intermediate levels (i.e., ~23 µmol/bottle of 1,2-DCA, or 22 mg/L; ~21 µmol/bottle of EDB, or 39 mg/L), decreases in 1,2-DCA became noticeable after day 103-178, while EDB showed a noticeable decrease after day 75-140. In medium controls with 1,2-DCA alone at high levels (96 µmol/bottle, or 92 mg/L), losses became noticeable after day 109. In the treatment with both 1,2-DCA and EDB present (i.e., ~90-109 µmol/bottle of 1,2-DCA, or 87-105 mg/L; ~56-57 µmol/bottle of EDB, or 103-105 mg/L), losses of 1,2-DCA became noticeable after day 35-53, while EDB showed no significant change during the 60 days of incubation.

Figure A-7.2 shows the results for the EDB water controls; water controls for 1,2-DCA were not prepared. The bottles were sampled only twice. Over the 80 days of incubation, no significant losses occurred.

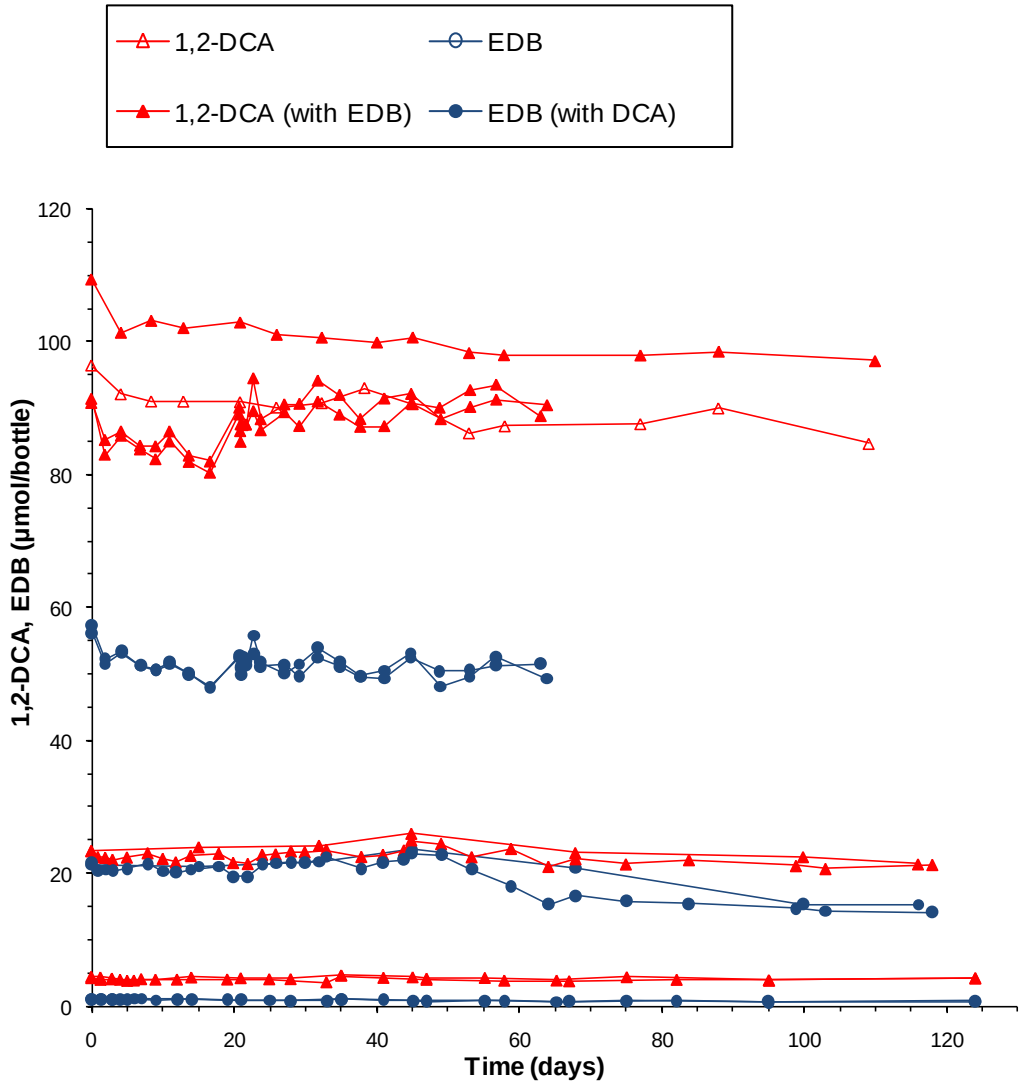


Figure A-7.1 Results for the mineral medium controls.

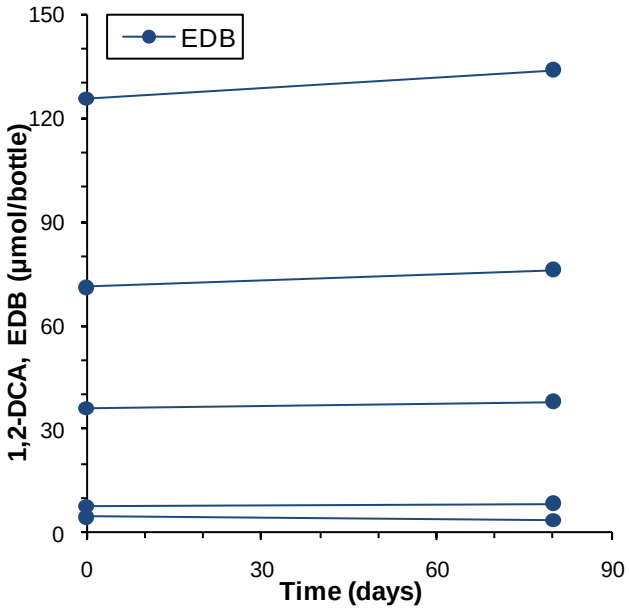


Figure A-7.2 Results for the EDB water controls.

Appendix-8: ComprehensiveResults for Maximum Growth Rates

In the Results section, under "High Concentrations of 1,2-DCA and EDB", representative results are presented for how the maximum growth rates were determined for treatments A, B, D and F (Figure 3.2a). The purpose of this section of the Appendixis to present the results for all of the bottles that were used to measure$\hat{\mu}$.

Average values and standard deviationsfor each treatment are provided in TablesA-8.1 to A-8.9.

Individual bottle results for treatment A are shown in Figures A-8.1 and A-8.2.

Individual bottle results for treatment B are shown in Figure A-8.3.

Individual bottle results for treatment C are shown in Figures A-8.4 and A-8.5.

Individual bottle results for treatment D are shown in Figure A-8.6.

Individual bottle results for treatment E are shown in Figure A-8.7.

Individual bottle results for treatment F are shown in Figures A-8.8 and A-8.9.

Table A-8.1 MaximumGrowth Rates for 1,2-DCA

Treatment	Substrate(s)/Culture	$\hat{\mu}$ (d^{-1})	No. of runs
A	DCA/DCA	0.5091 ± 0.0662^{a}	8
B	DCA+EDB/DCA	0.5763 ± 0.0918	4
C	DCA/EDB	0.5346 ± 0.0833	6
D	DCA+EDB/EDB	0.1873 ± 0.0231	2

[a]Standard deviation.

Table A-8.2 MaximumGrowth Rates for EDB

Treatment	Substrate(s)/Culture	$\hat{\mu}$ (d^{-1})	No. of runs
B	EDB+DCA/DCA	0.3537 ± 0.0209^{a}	5
D	EDB+DCA/EDB	0.4020 ± 0.0483	4
E	EDB/DCA	0.5427 ± 0.0642	5
F	EDB/EDB	0.4539 ± 0.0697	6

[a]Standard deviation.

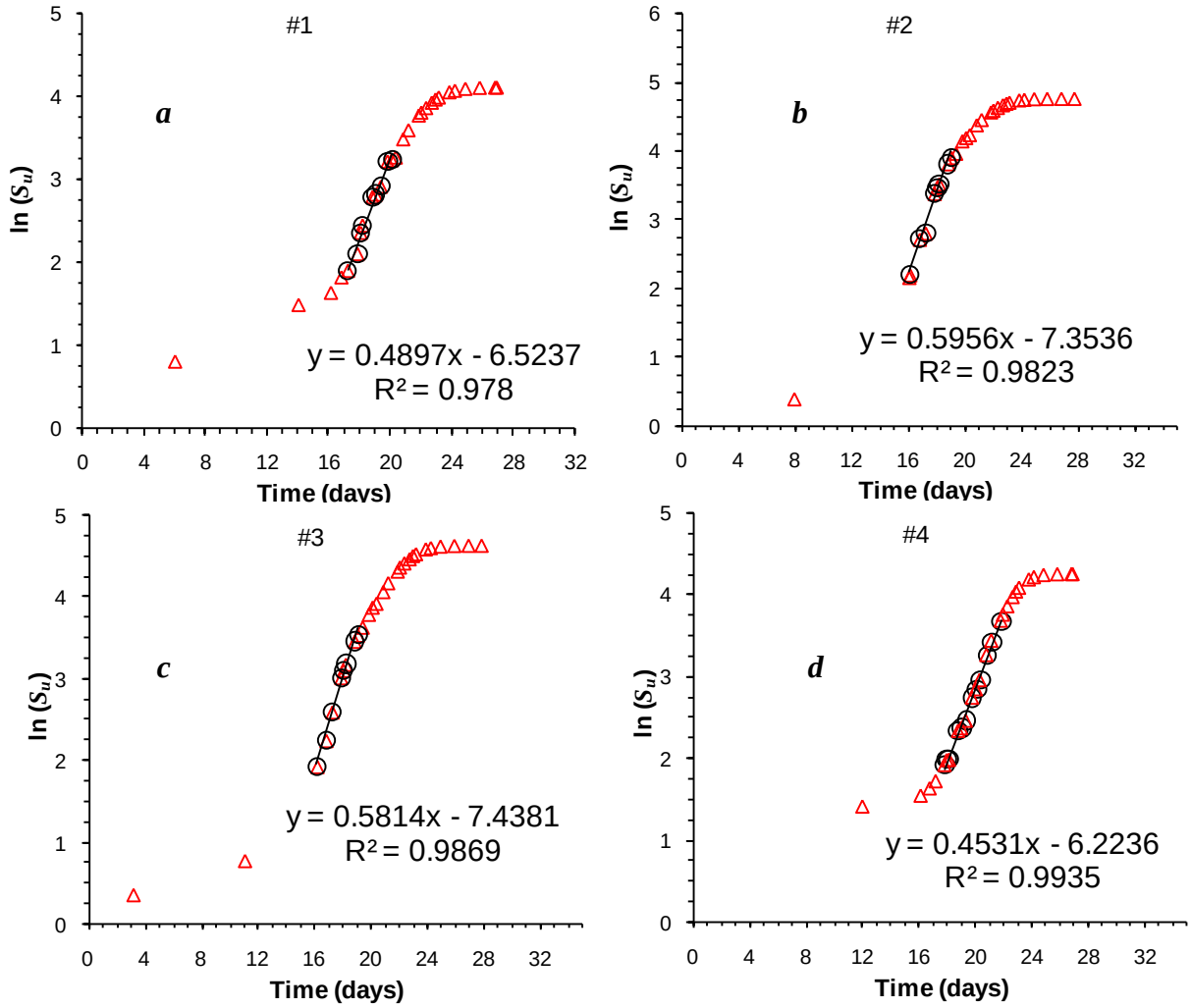


Figure A-8.1 Results for 1,2-DCA in treatment A, (***a***) bottle #1; (***b***) bottle #2; (***c***) bottle #3; and (***d***) bottle #4.

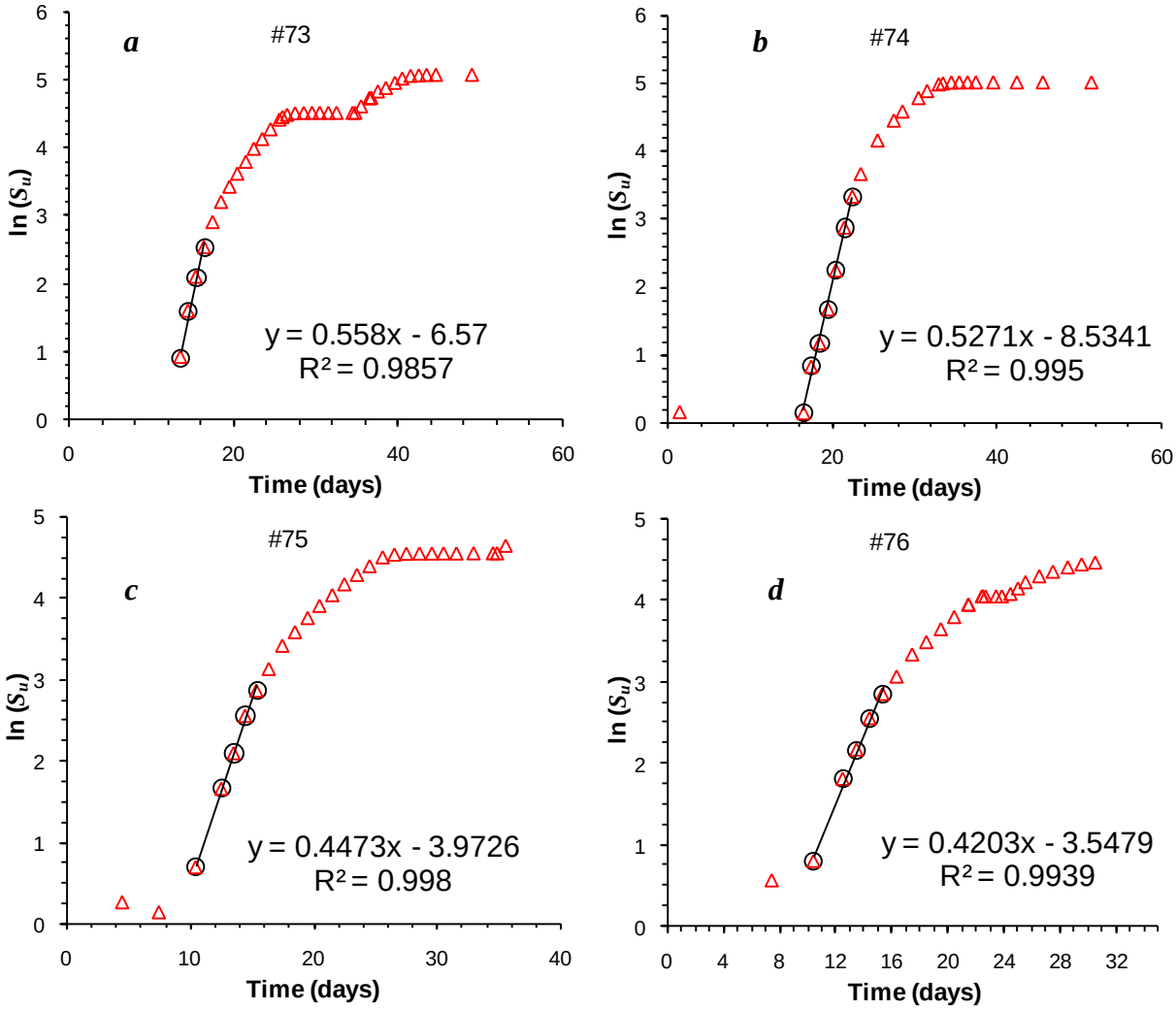


Figure A-8.2 Results for 1,2-DCA in treatment A, (***a***) bottle #73; (***b***) bottle #74; (***c***) bottle #75; and (***d***) bottle #76.

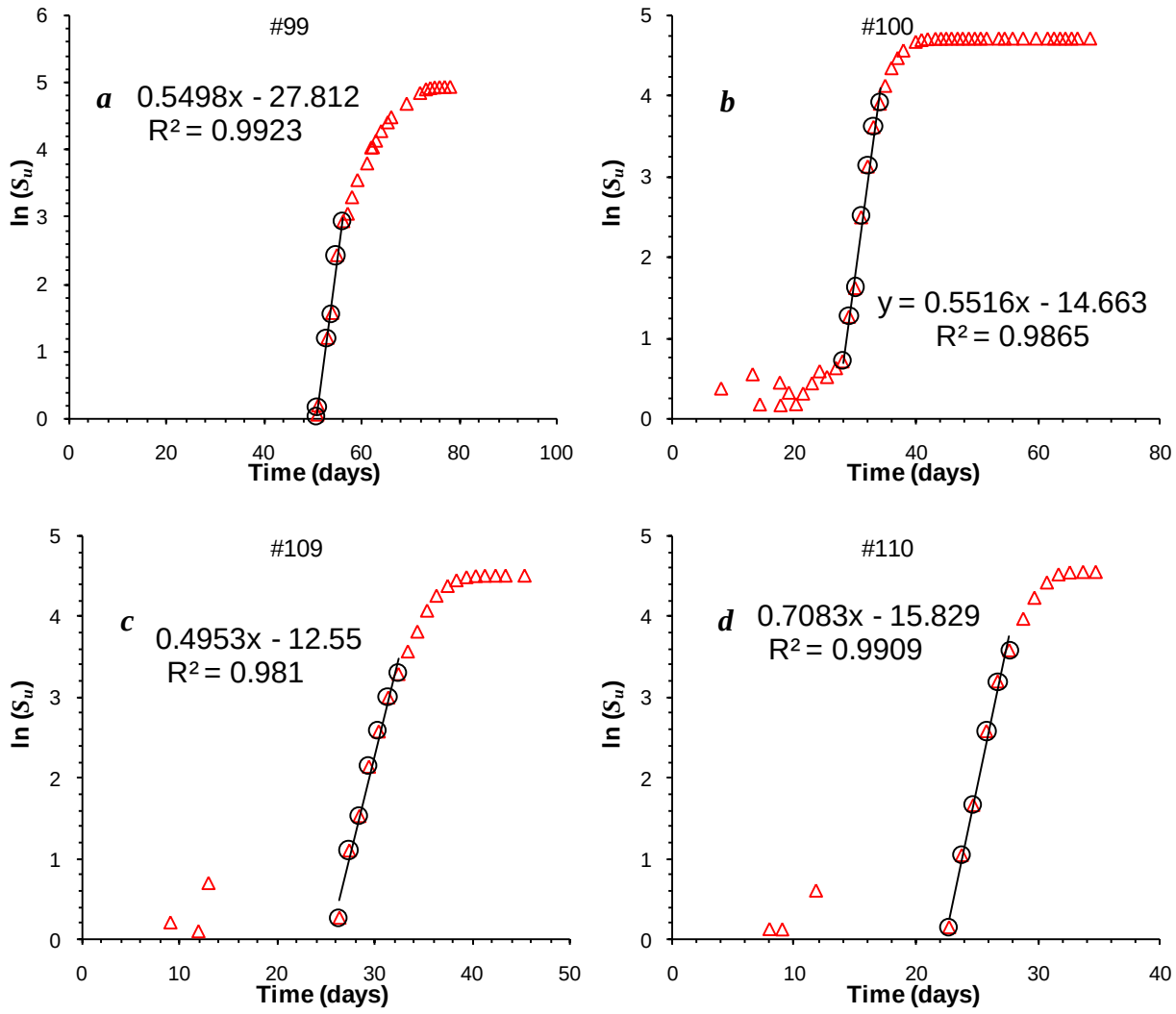


Figure A-8.3 Results for 1,2-DCA in treatment B, (***a***) bottle #99; (***b***) bottle #100; (***c***) bottle #109; and (***d***) bottle #110.

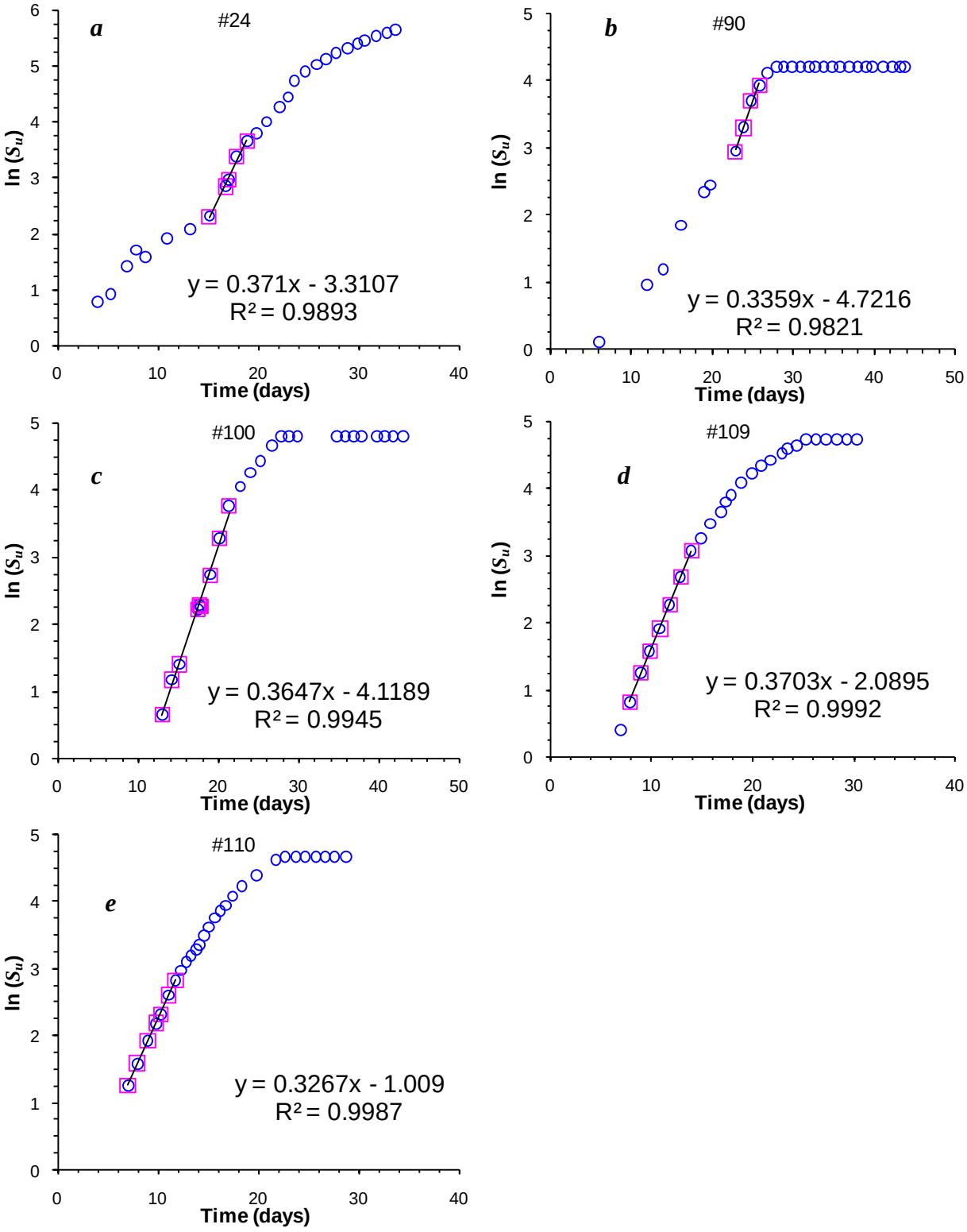


Figure A-8.4 Results for EDB in treatment B, (***a***) bottle #24; (***b***) bottle #90; (***c***) bottle #10; (***d***) bottle #109; and (***e***) bottle #110.

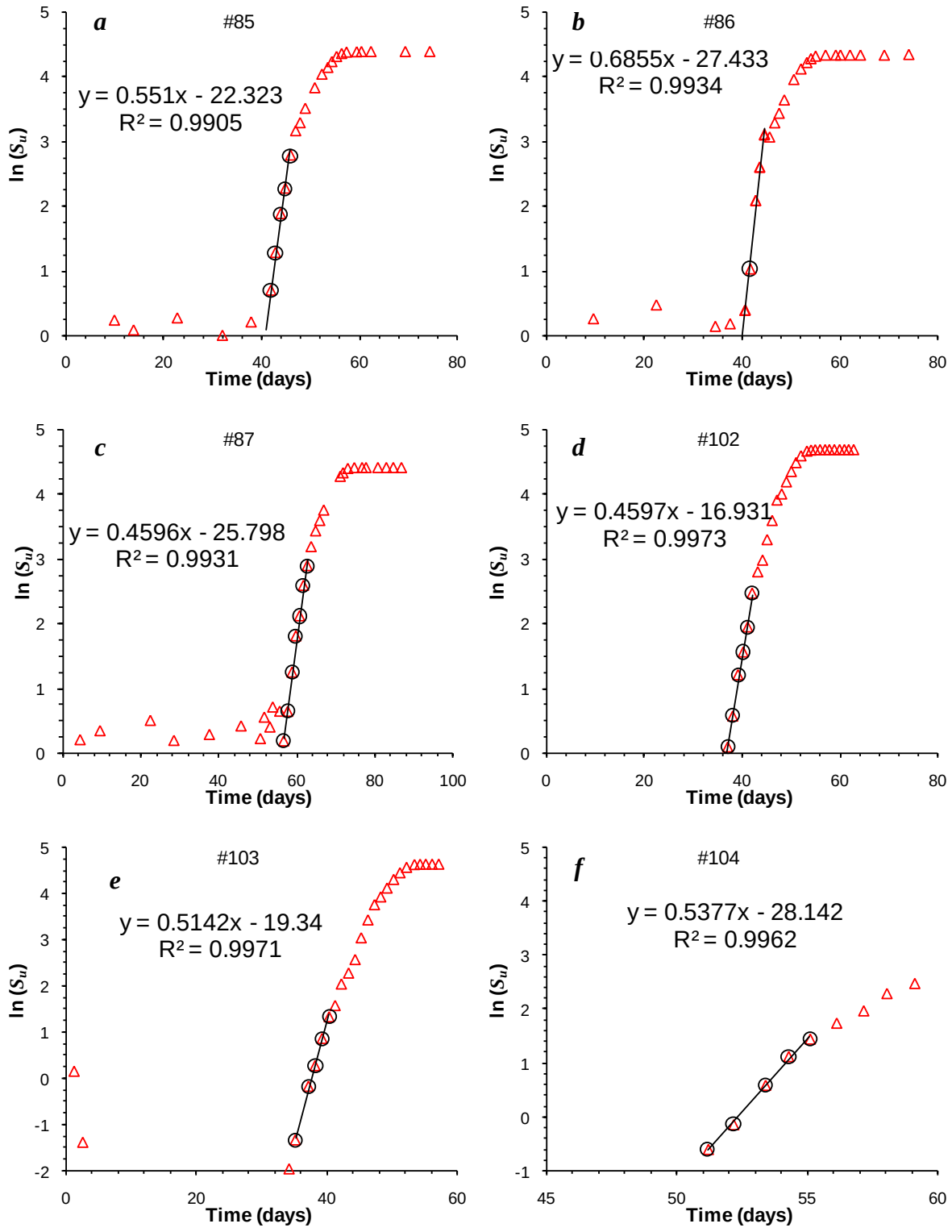


Figure A-8.5 Results for 1,2-DCA in treatment C, (***a***) bottle #85; (***b***) bottle #86; (***c***) bottle #87; (***d***) bottle #102; (***e***) bottle #103; and (***f***) bottle #104.

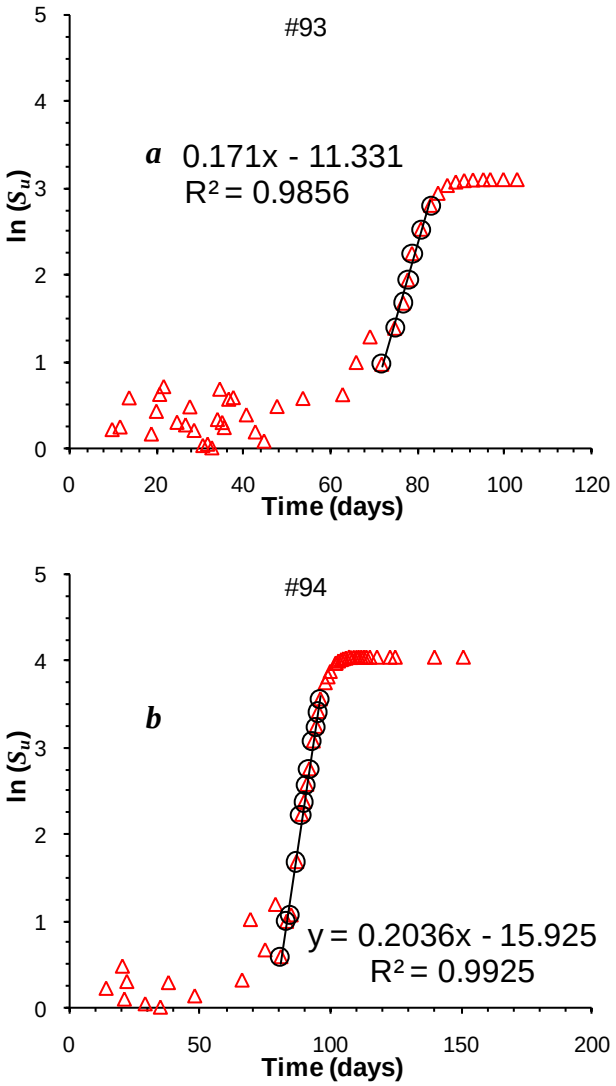


Figure A-8.6 Results for 1,2-DCA in treatment D, (***a***) bottle #96; and (***b***) bottle #94.

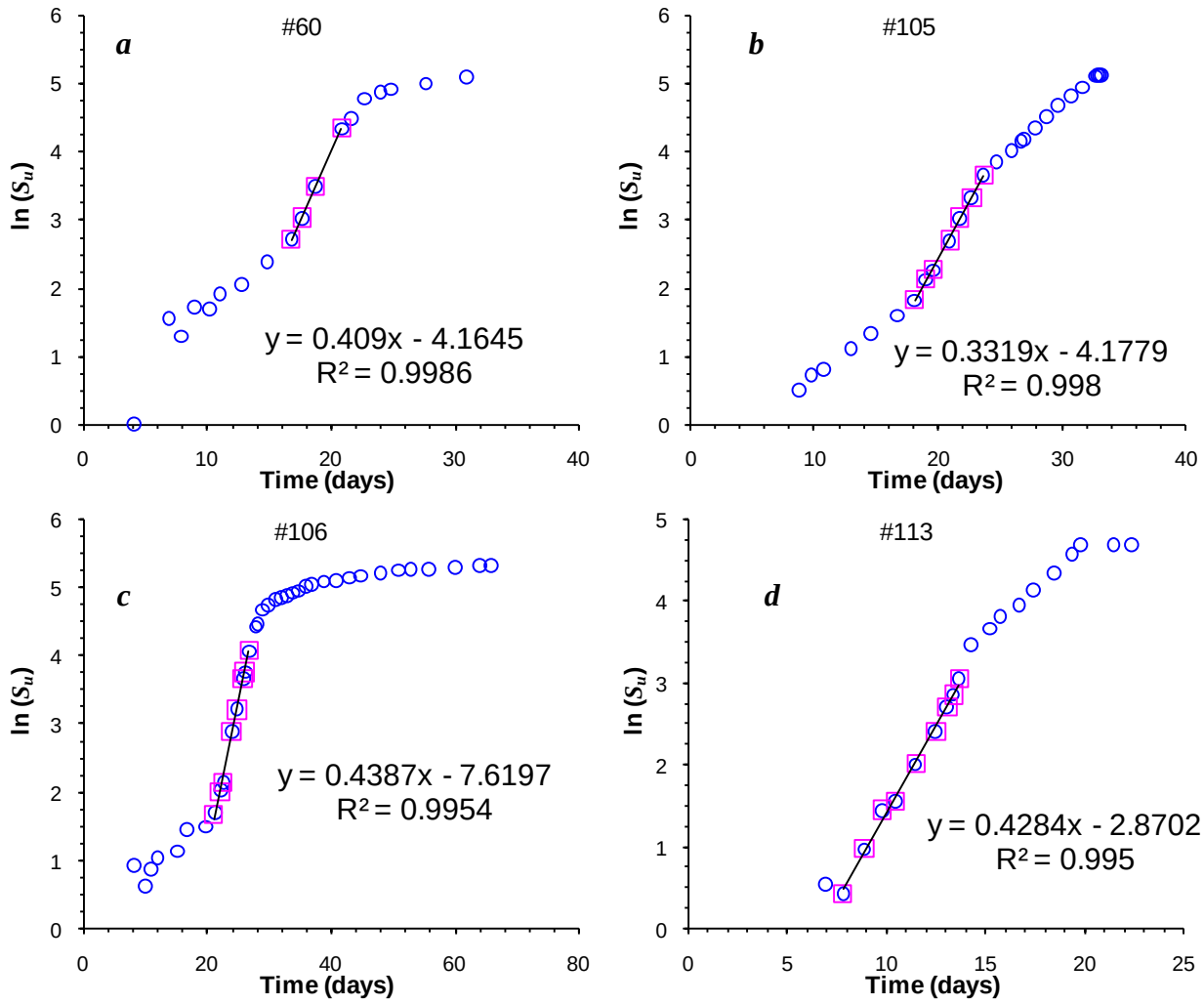


Figure A-8.7 Results for EDB in treatment D, (***a***) bottle #60; (***b***) bottle #105; (***c***) bottle #106; and (***d***) bottle #113.

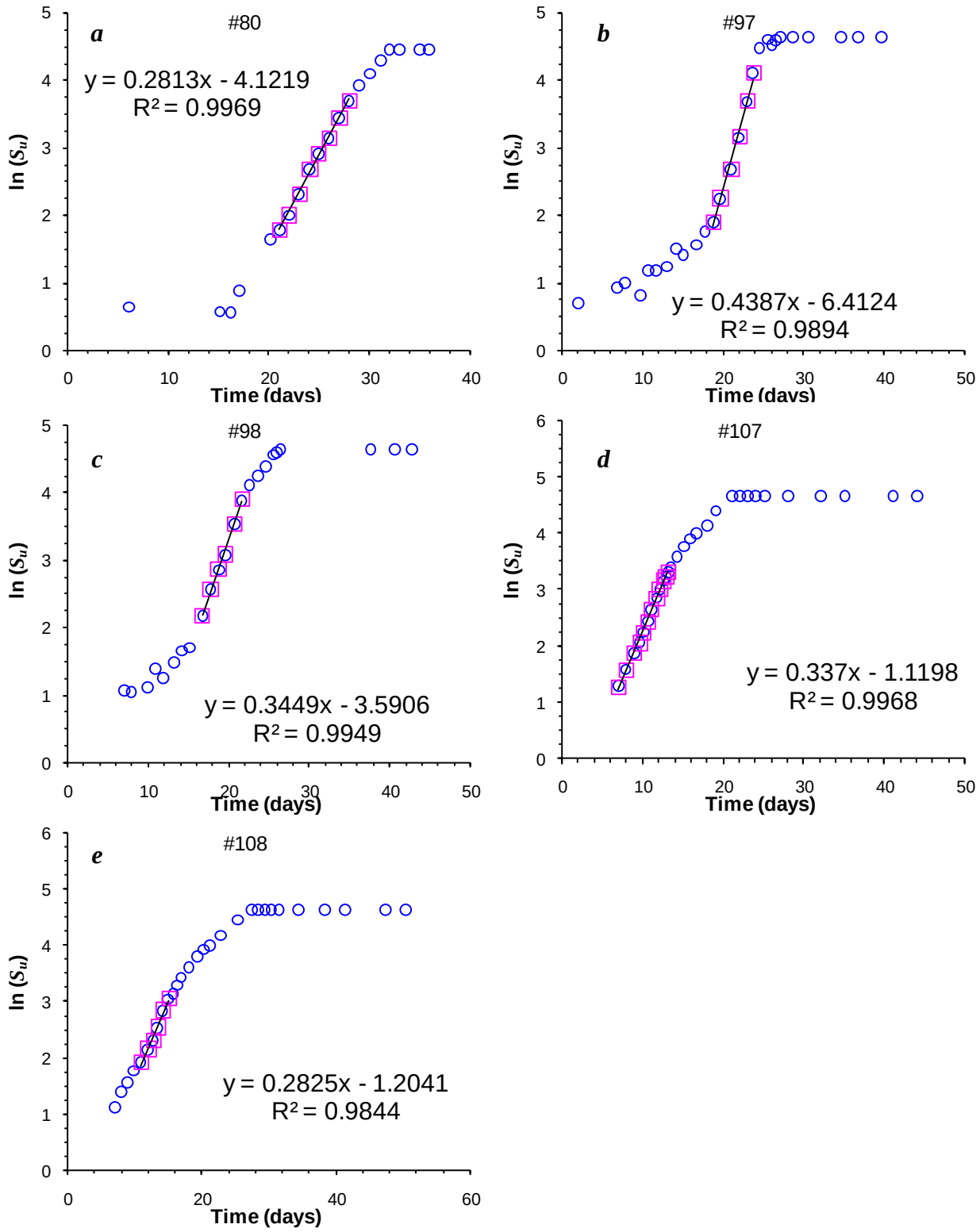


Figure A-8.8 Results for EDB in treatment E, (***a***) bottle #80; (***b***) bottle #97; (***c***) bottle #98; (***d***) bottle #107; and (***e***) bottle #108.

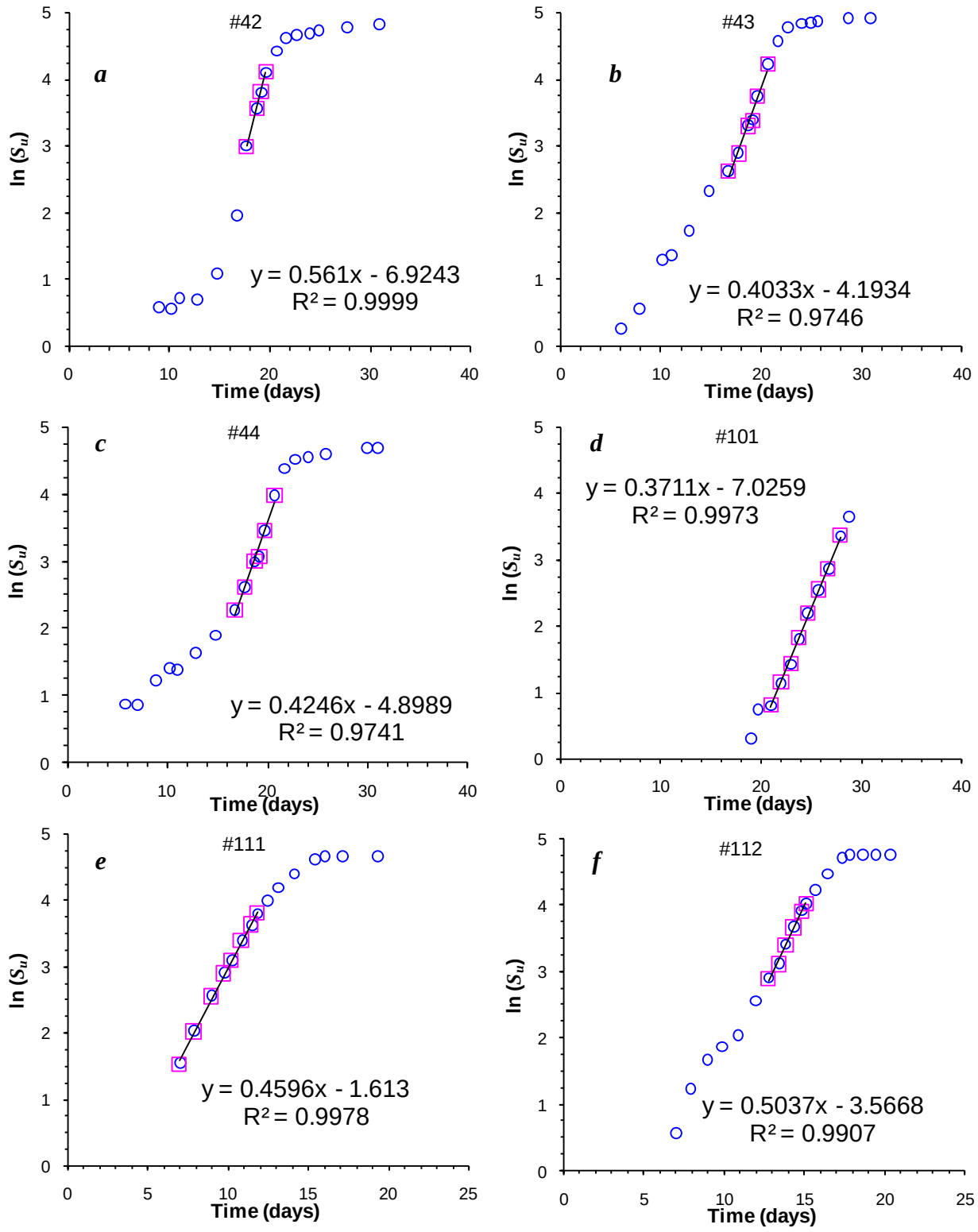


Figure A-8.9 Results for EDB in treatment F, (***a***) bottle #42; (***b***) bottle #43; (***c***) bottle #44; (***d***) bottle #101; (***e***) bottle #111; and (***f***) bottle #112.

Appendix-9: Comprehensive Results for K_S, S_t and $\hat{r}$

In the Results section, under "High Concentrations of 1,2-DCA and EDB", representative results are presented for how the half saturation coefficients, transition concentrations, and maximum substrate utilization rates were determined for treatments A, B, D and F (Figure 3.2, panels b and c). The purpose of this section of the Appendixis to present the results for all of the bottles that were used to measure K_S, S_t, and $\hat{r}$.

Average values and standard deviations for each treatment are provided in Table A-9.1 to Table A-9.12.

Individual bottle results for treatment A are shown in Figures A-9.1 and A-9.2.

Individual bottle results for treatment B are shown in Figures A-9.3 and A-9.4.

Individual bottle results for treatment C are shown in Figures A-9.5 and A-9.6.

Individual bottle results for treatment D are shown in FiguresA-9.7 and A-9.8.

Individual bottle results for treatment E are shown in FiguresA-9.9 and A-9.10.

Individual bottle results for treatment F are shown in Figures A-9.11 and A-9.12.

Table A-9.1 Results for K_S, S_t and $\hat{r}$

Treatment	$\hat{r}$ (mg/L/d)	K_s (mg/L)	S_t (µg/L)	No. of runs
A	13.9 ± 4.4[a]	8.439 ± 0.044[a]	38.28 ± 6.29[a]	11
B	15.4 ± 7.8	15.679 ± 0.064	28.84 ± 4.29	6
C	10.9 ± 3.2	5.724 ± 0.044	12.85 ± 3.82	9
D	6.0 ± 1.0	12.366 ± 0.027	127.48 ± 5.19	2
B	11.0 ± 4.7	8.78E-03 ± 0.0132	0.691 ± 0.844	6
D	20.1 ± 3.3	1.54E-02 ± 0.0059	9.184 ± 2.842	5
E	9.5 ± 3.4	7.75E-05 ± 0.0031	5.264 ± 3.379	8
F	17.3 ± 4.7	8.37E-01 ± 0.0157	0.495 ± 0.519	8

[a]Standard deviation.

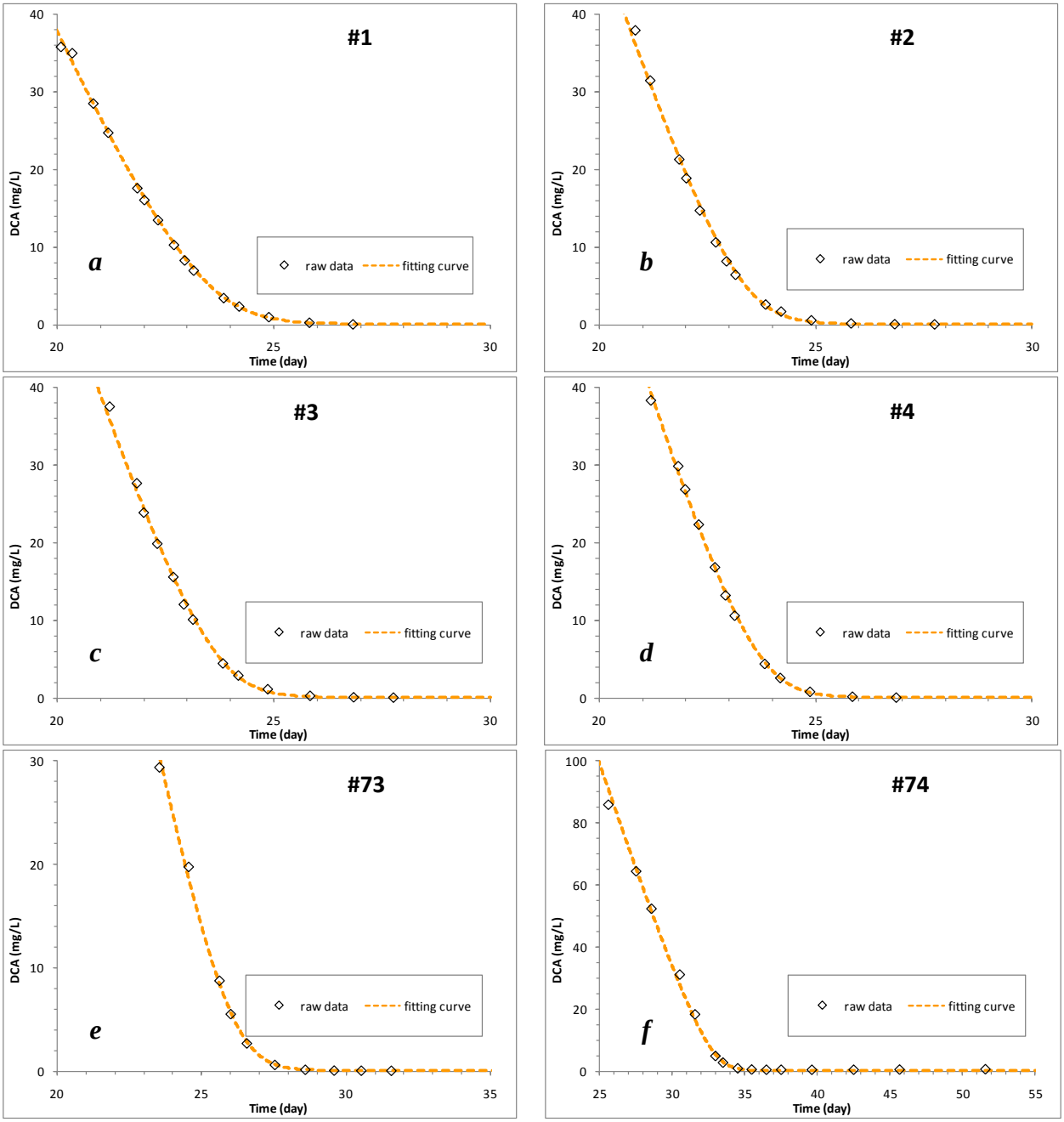


Figure A-9.1 Results for 1,2-DCA in treatment A, (***a***) bottle #1; (***b***) bottle #2; (***c***) bottle #3; (***d***) bottle #4; (***e***) bottle #73 and (***f***) bottle #74.

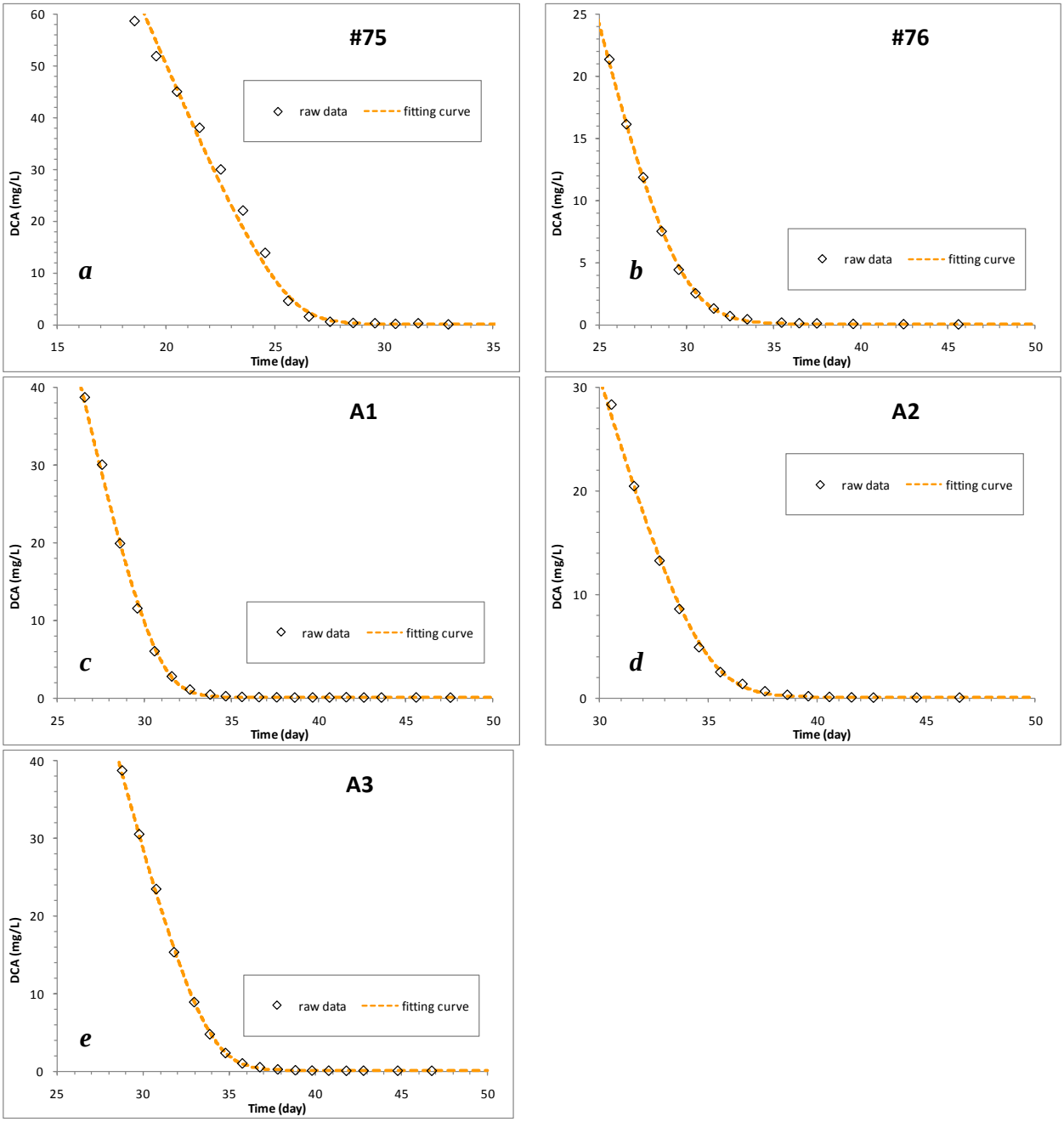


Figure A-9.2 Results for 1,2-DCA in treatment A, (***a***) bottle #75; (***b***) bottle #76; (***c***) bottle #A1; (***d***) bottle #A2 and (***e***) bottle #A3.

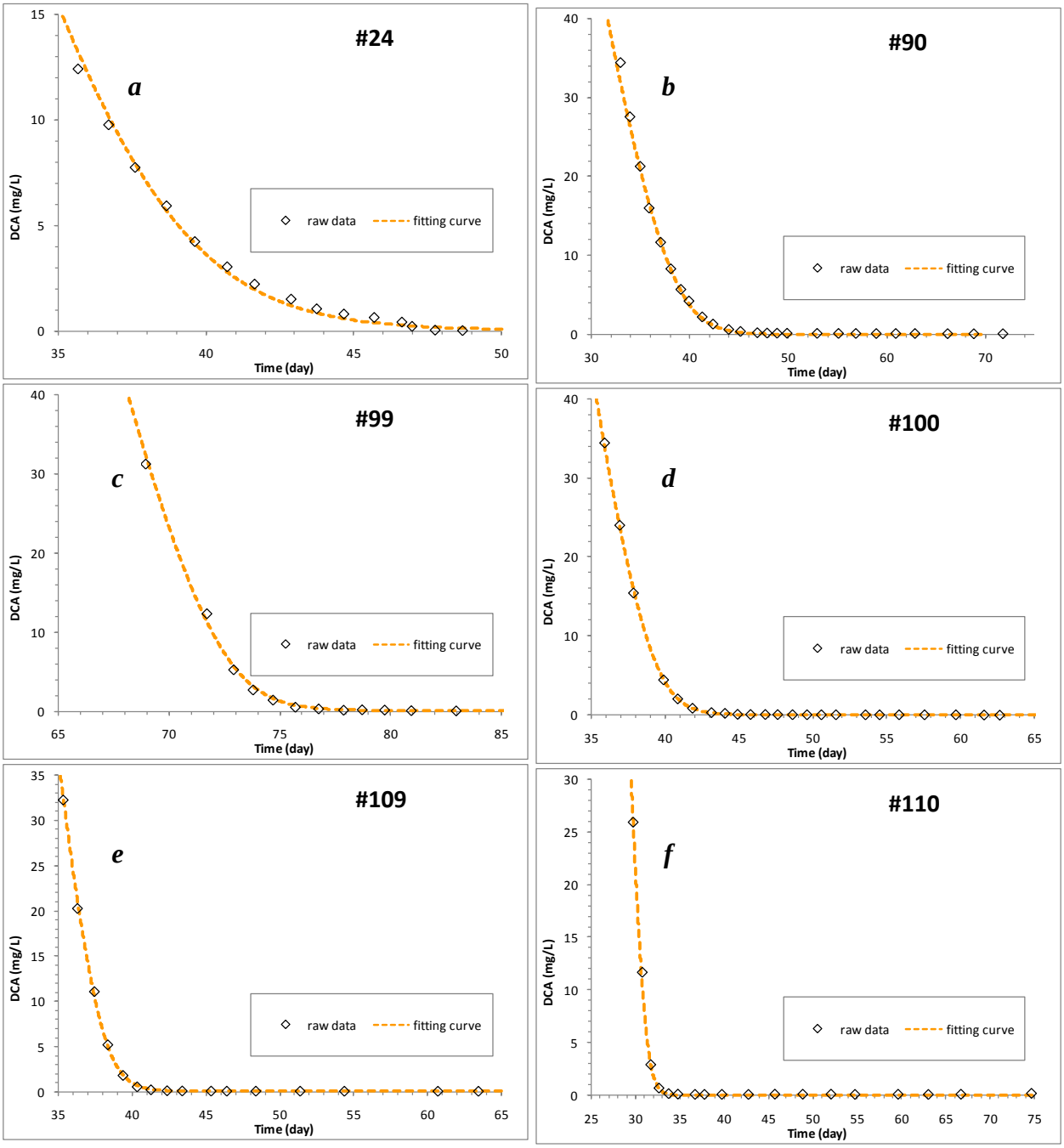


Figure A-9.3 Results for 1,2-DCA in treatment B, (***a***) bottle #24; (***b***) bottle #90; (***c***) bottle #99; (***d***) bottle #100; (***e***) bottle #109 and (***f***) bottle #110.

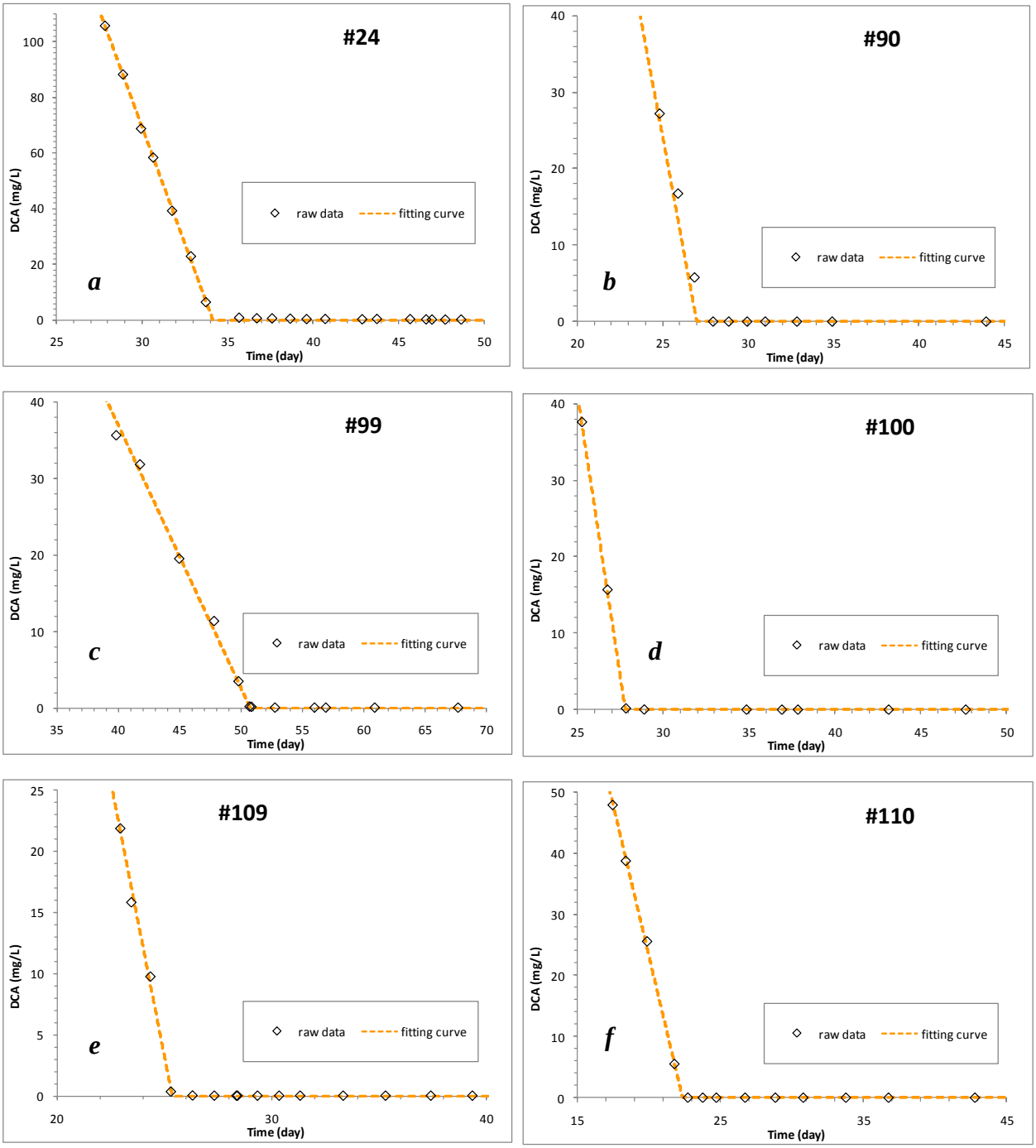


Figure A-9.4 Results for EDB in treatment B, (***a***) bottle #24; (***b***) bottle #90; (***c***) bottle #99; (***d***) bottle #100; (***e***) bottle #109 and (***f***) bottle #110.

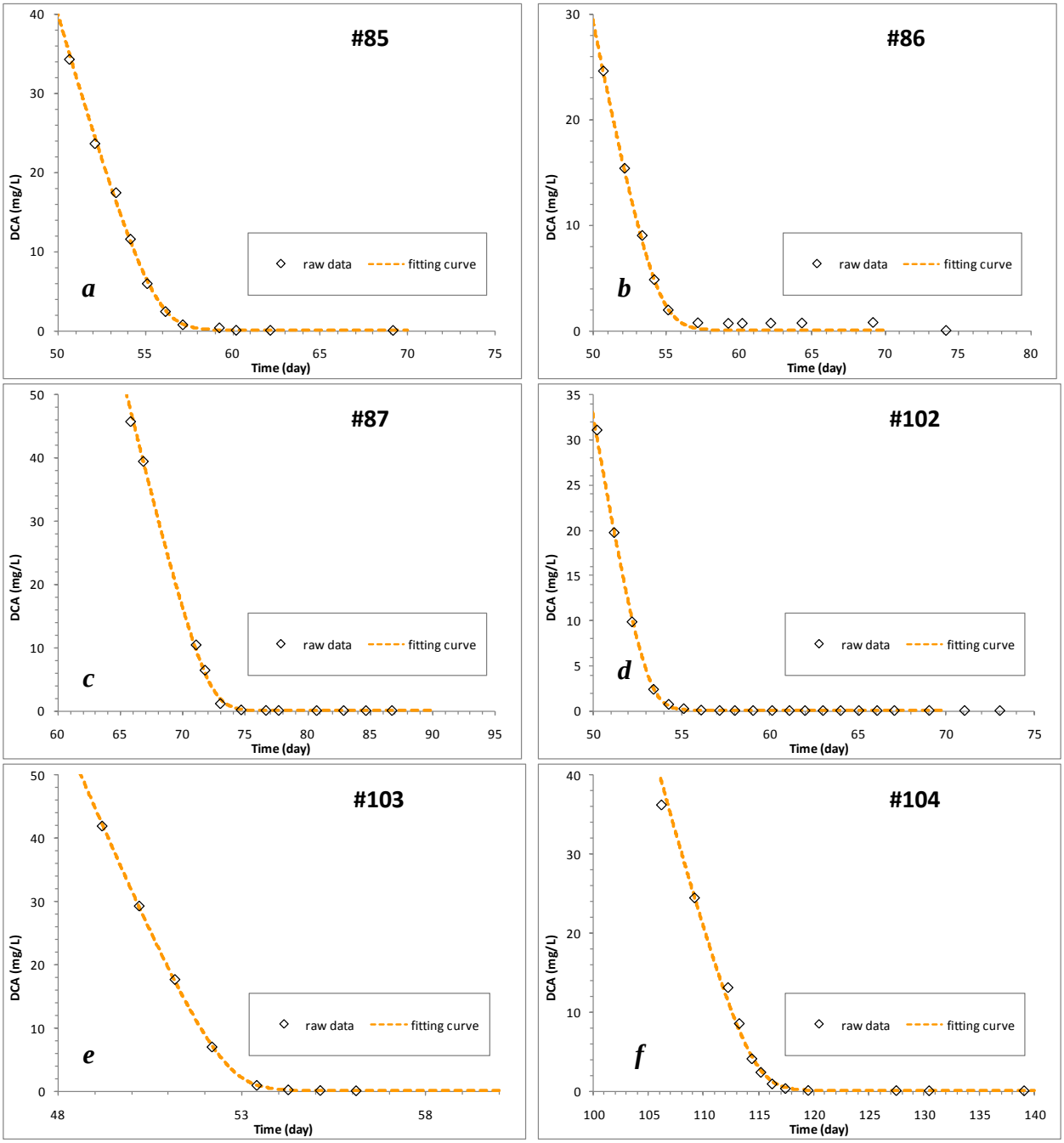


Figure A-9.5 Results for 1,2-DCA in treatment C, (***a***) bottle #85; (***b***) bottle #86; (***c***) bottle #87; (***d***) bottle #102; (***e***) bottle #103 and (***f***) bottle #104.

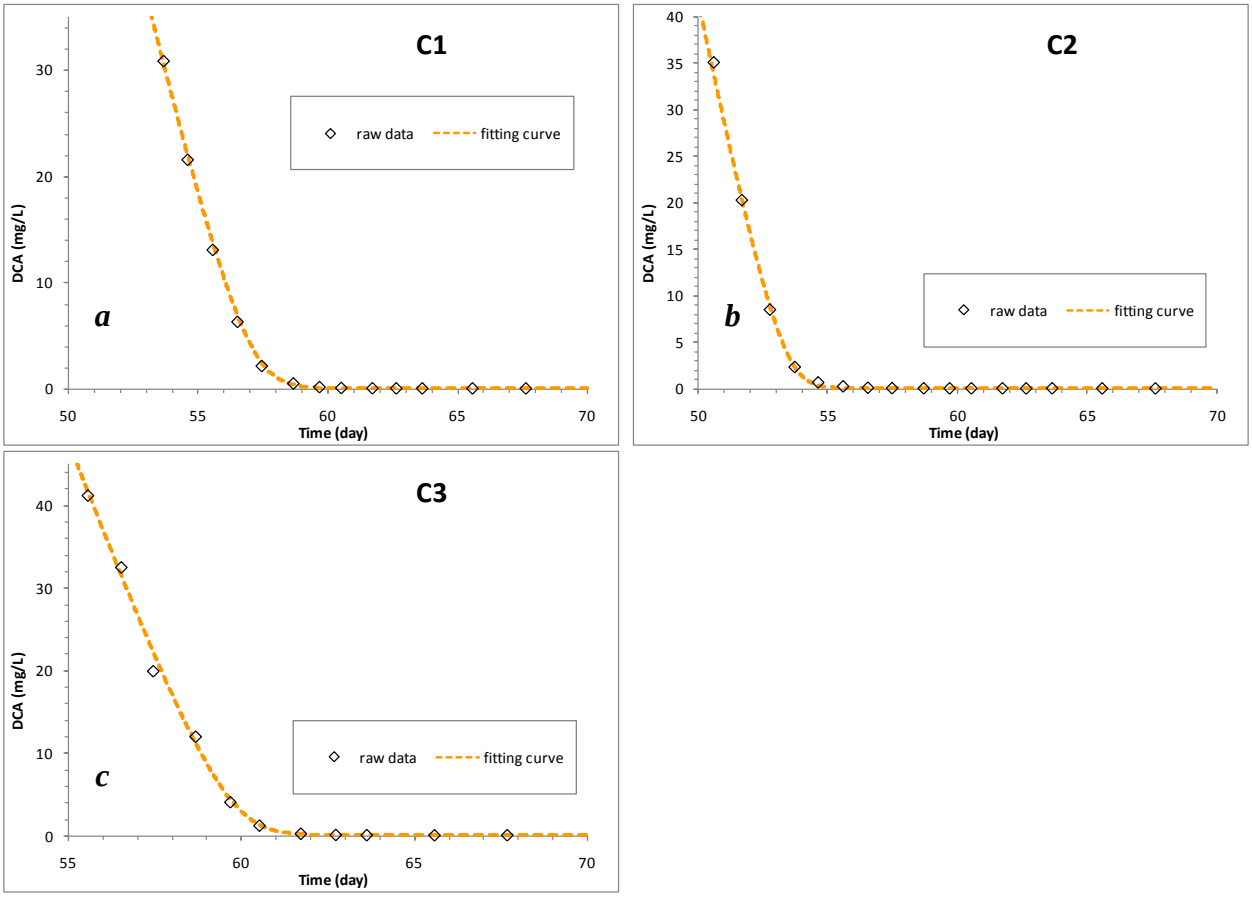


Figure A-9.6 Results for 1,2-DCA in treatment C, (***a***) bottle #C1; (***b***) bottle #C2 and (***c***) bottle #C3.

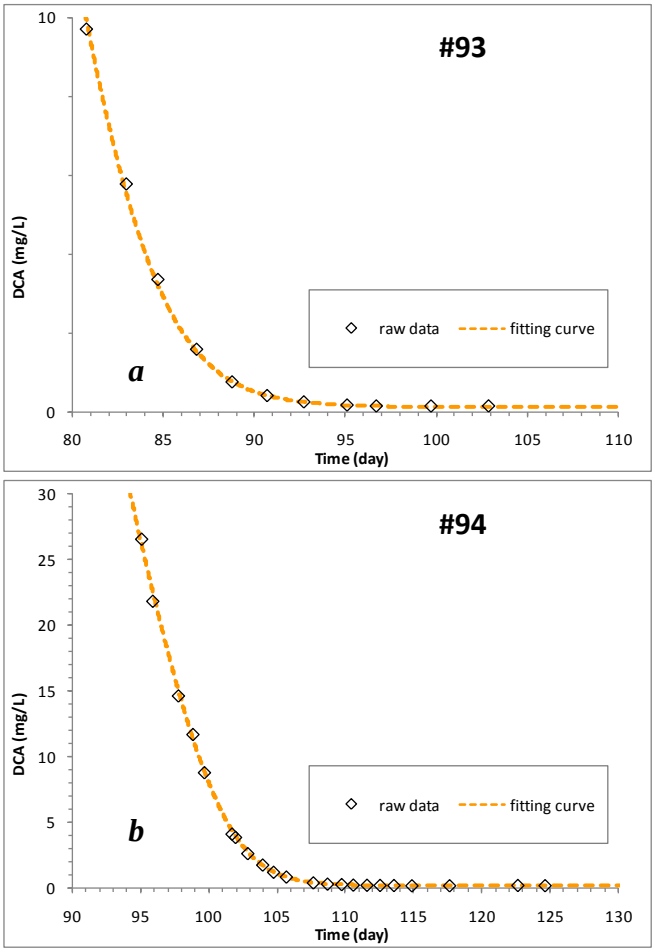


Figure A-9.7 Results for 1,2-DCA in treatment D, (***a***) bottle #93 and (***b***) bottle #94.

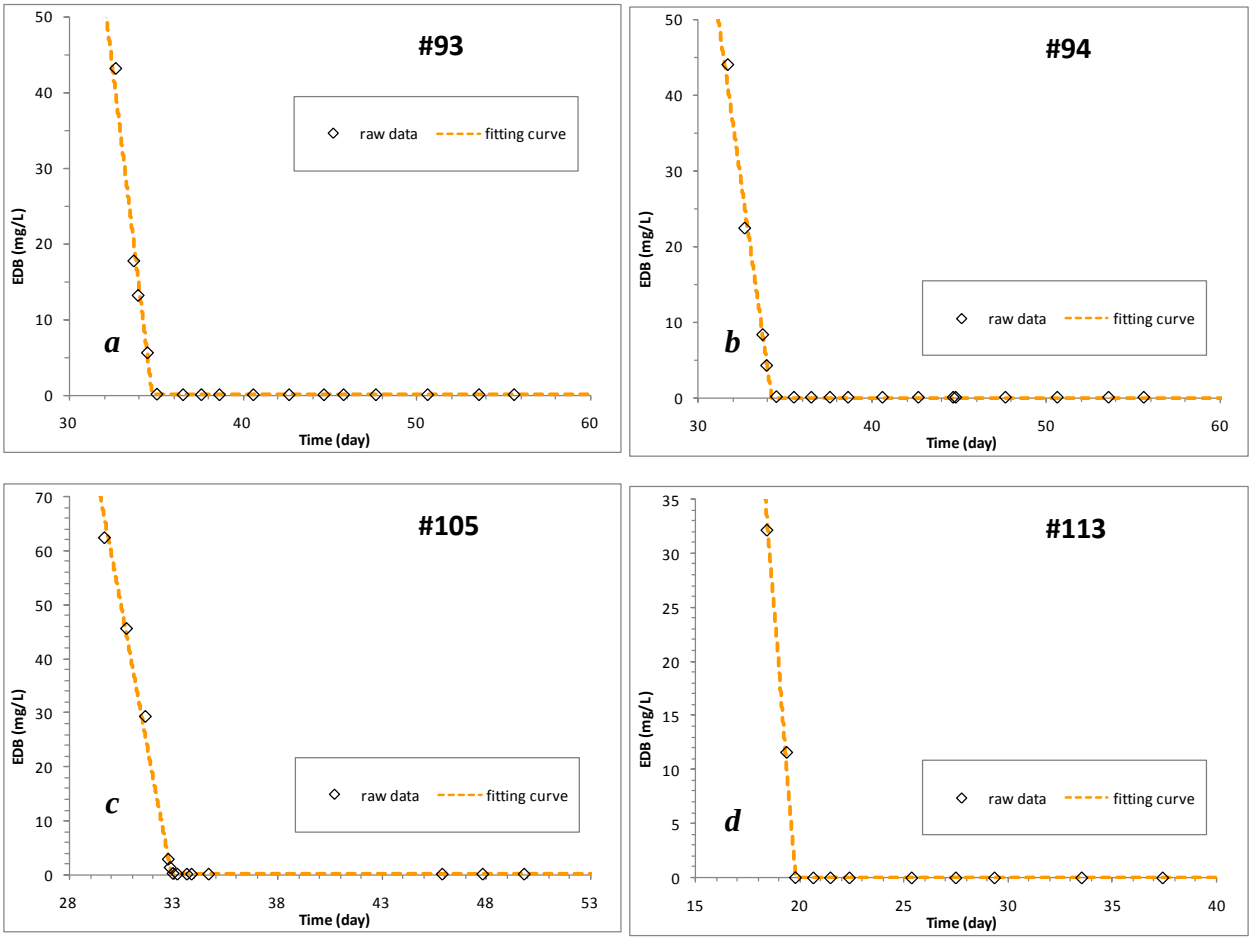


Figure A-9.8 Results for EDB in treatment D, (***a***) bottle #93; (***b***) bottle #94; (***c***) bottle #105 and (***d***) bottle #113.

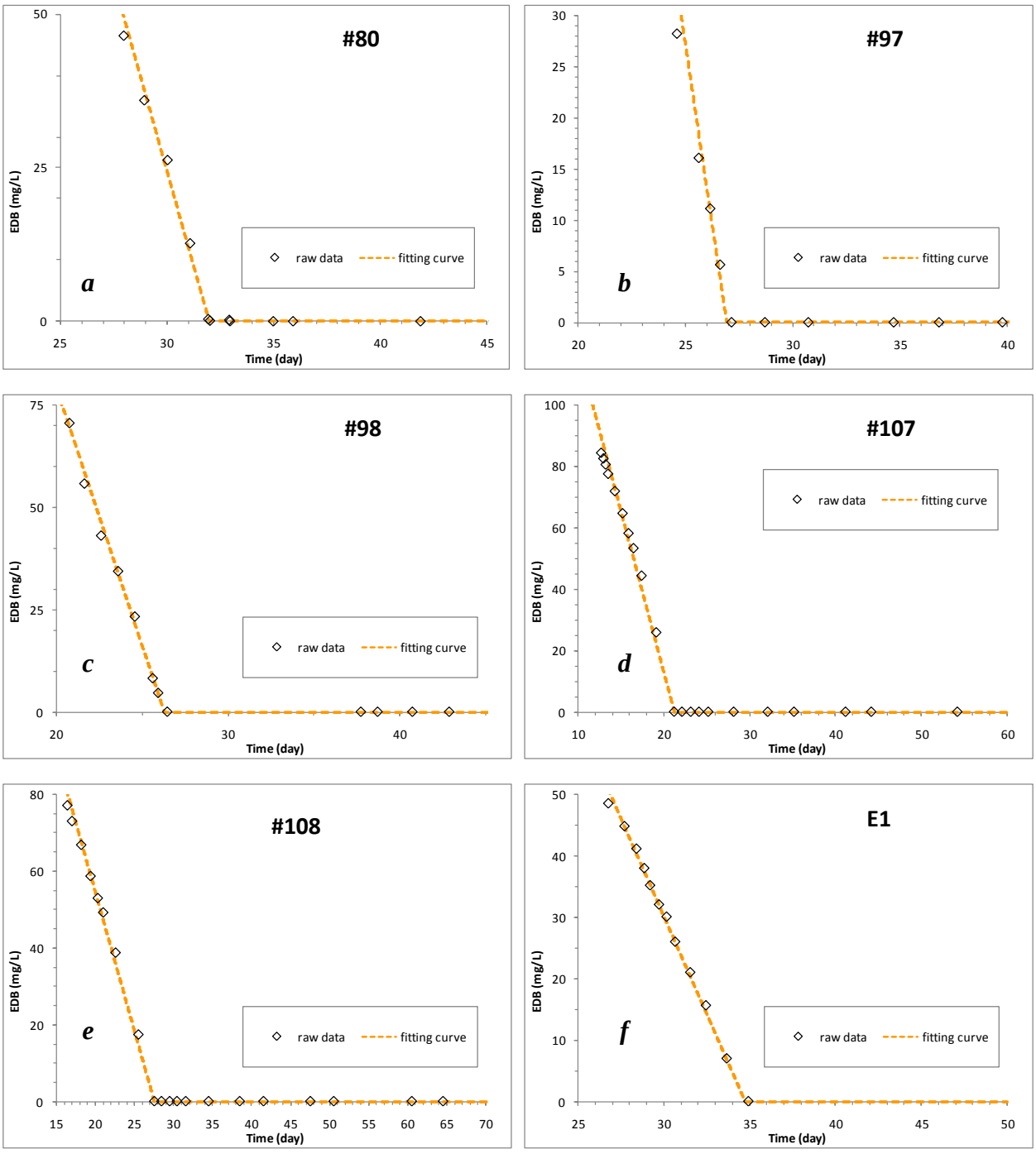


Figure A-9.9 Results for EDB in treatment E, (***a***) bottle #80; (***b***) bottle #97; (***c***) bottle #98; (***d***) bottle #107; (***e***) bottle #108 and (***f***) bottle #E1.

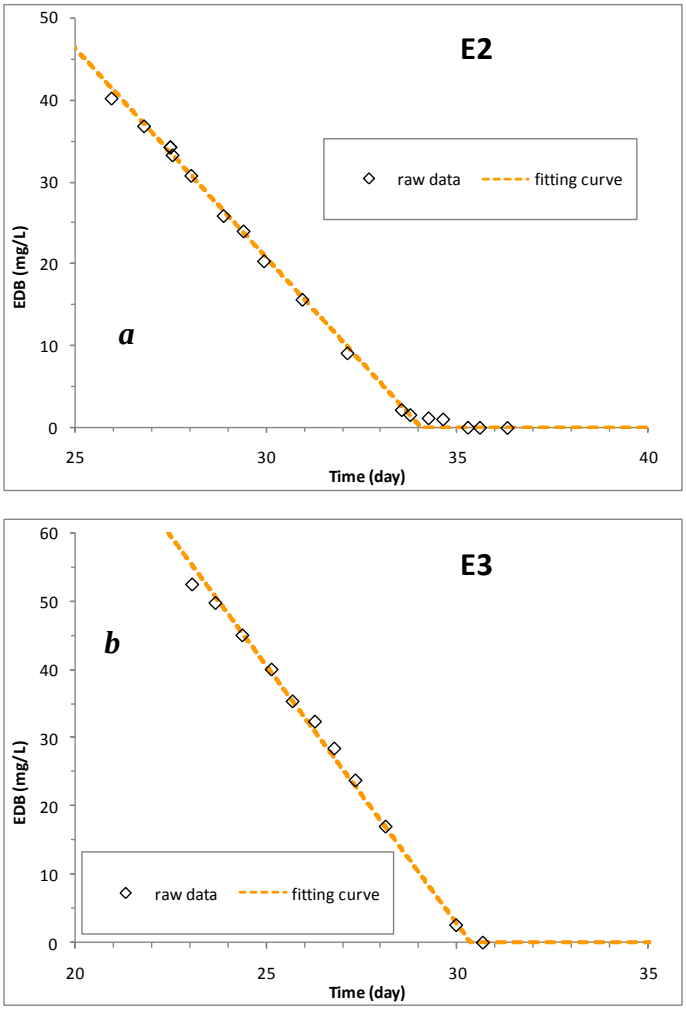


Figure A-9.10 Results for EDB in treatment E, (***a***) bottle #E2 and (***b***) bottle #E3.

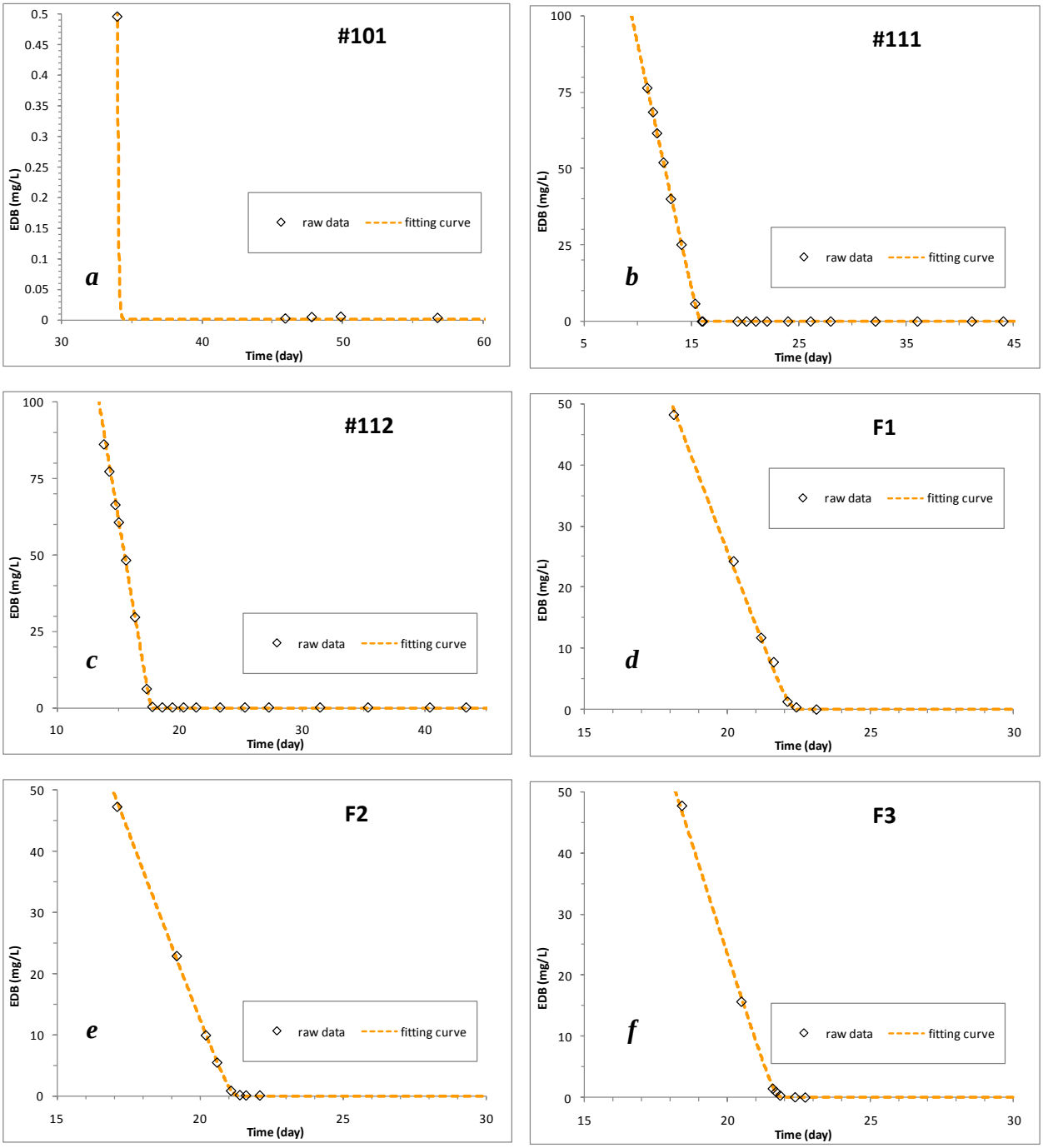


Figure A-9.11 Results for EDB in treatment F, (***a***) bottle #101; (***b***) bottle #111; (***c***) bottle #112; (***d***) bottle #F1; (***e***) bottle #F2 and (***f***) bottle #F3.

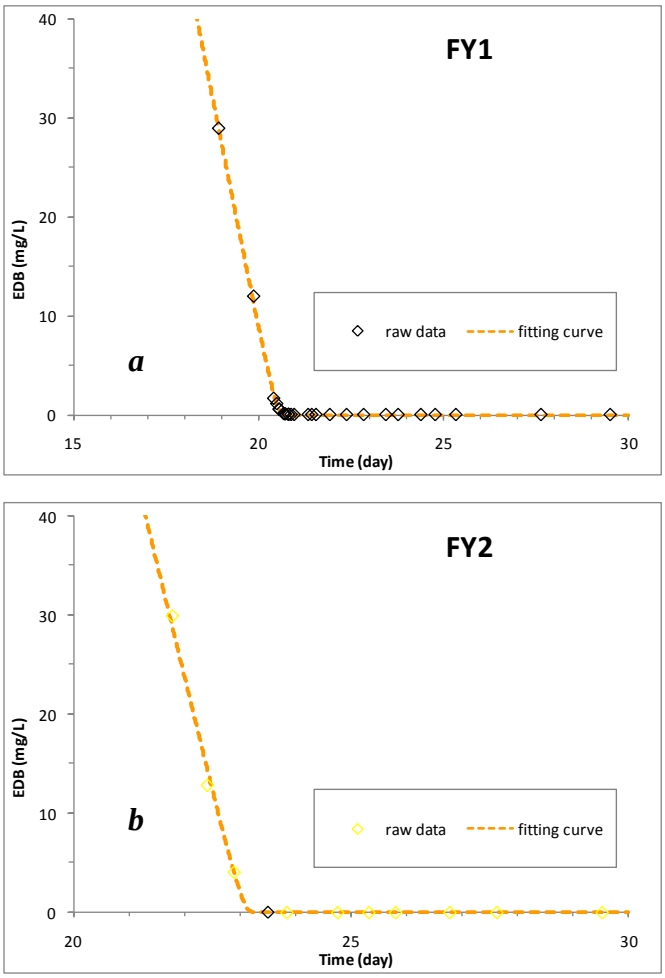


Figure A-9.12 Results for EDB in treatment F, (***a***) bottle #FY1 and (***b***) bottle #FY2.

Appendix-10: Effect of S_t on K_S

In the Discussion section, mention is made about the effect of includingtransition concentrations (S_t) on the fitting results of Monod half saturation coefficients (K_S). This section provides a comparison betweenK_S values when S_t was and was not included. K_S and its coefficient of variation (cv = standard deviation divided by the value) are provided in Table A-10.1.

Table A-10.1 Effect of Including S_t in the Monod Equation on the Fitting Results for K_S

	1,2-DCA				EDB			
	with S_t		without S_t		with S_t		without S_t	
Treatment	K_S	**cv**	K_S	**cv**	K_S	**cv**	K_S	**cv**
A	8.439	0.52%	8.80	0.54%	-	-	-	-
B	15.679	0.41%	17.3	0.37%	8.78E-03	150%	3.35E-05	39487%
C	5.724	0.77%	5.73	0.80%	-	-	-	-
D	12.366	0.22%	22.6	0.60%	1.54E-02	38.5%	4.57E-05	10373%
E	-	-	-	-	7.75E-05	3949%	3.22E-05	8843%
F	-	-	-	-	8.37E-01	2%	8.78E-01	2%

Appendix-11: Biomass Levels Based on Protein Concentrations

Mention was made about measurement of biomass concentrations based on protein, both in the Materials and Method section under “Batch Kinetics at High Concentrations”, and in the Results section under “High concentrations of 1,2-DCA and EDB”. This appendix provides the results for the protein analyses.

In order to measure protein over time, aseparate setof bottles wasprepared with the same headspace versus aqueous phase volume ratio as used in the serum bottles, but with the bottle size increasedto 1 liter. This allowed for frequent sample removal for protein measurementwithoutsignificantly affecting the liquid-to-headspace ratio in the bottles. The amounts of inoculum and substrates added were increased proportionately to the serum bottles, andthe same feeding procedure for 1,2-DCA, EDB and lactate was followed. Duplicate bottles were prepared for treatments A, C, E, and F. Protein was measured as described in section 2.2.

The concentration of protein for each treatment is shown in Figure A-11.1, along with the 1,2-DCA and EDB data that were used to determine K_S, S_t and $\hat{r}$. As indicated in the test, there was no significant change in protein concentrations during this time interval, lending support for the assumption the biomass concentration had reached a maximum.

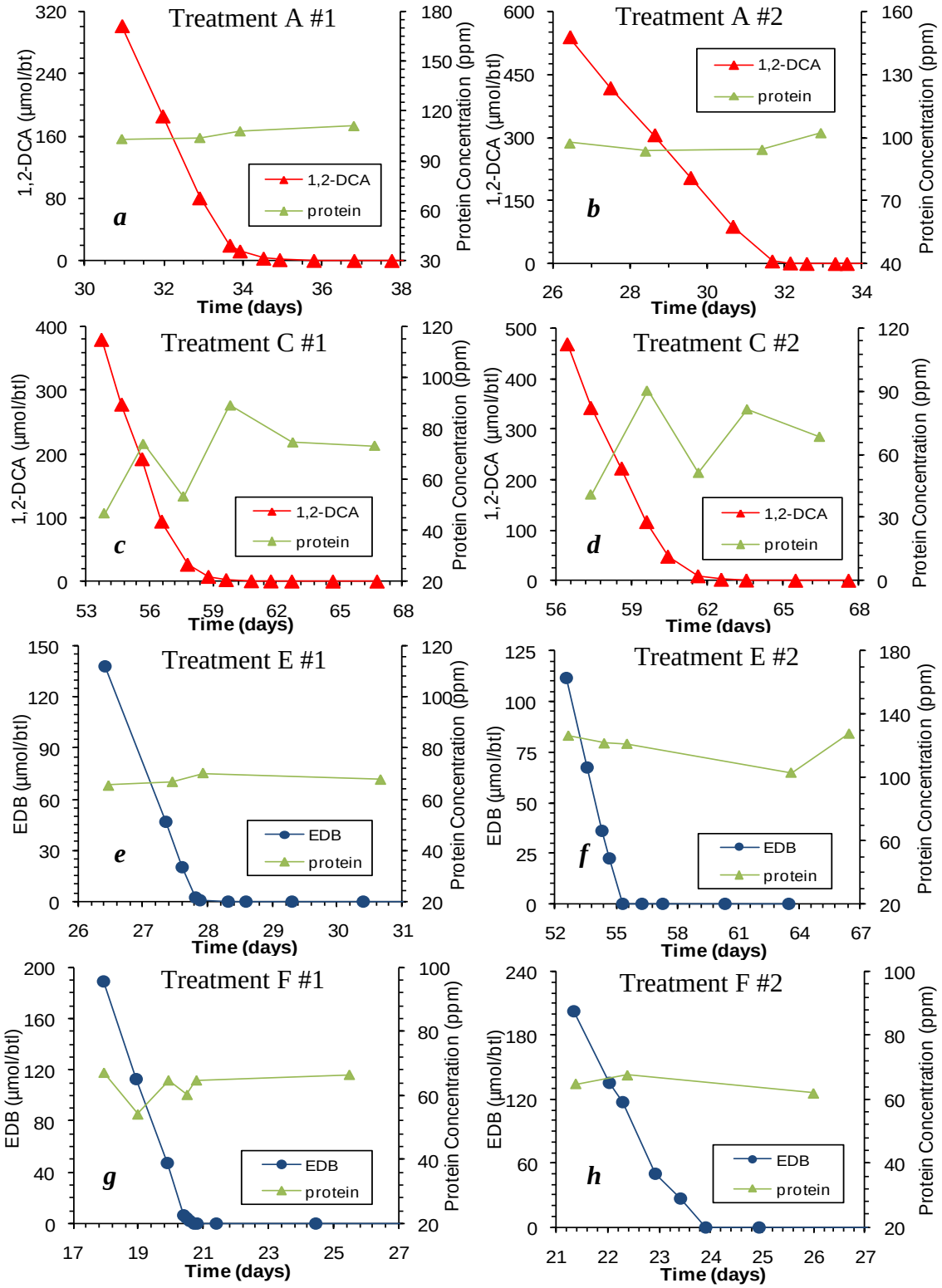


Figure A-11.1 Protein results for (***a***) treatment A, bottle #1; (***b***) treatment A, bottle #2; (***c***) treatment C, bottle #1; (***d***) treatment C, bottle #2; (***e***) treatment E, bottle #1; (***f***), treatment E, bottle #2; (***g***) treatment F, bottle #1; and (***h***) treatment F, bottle #2.

Appendix-12: Effect of Low EDB Levels on the Rate of 1,2-DCA Dechlorination

In the manuscript, results for only one of the duplicates in the test of "Effect of low EDB levels on 1,2-DCA" are shown in Figure 3.4. The purpose of this Appendix is to provide the results for the duplicate bottles. As shown in Figure A-12.1, the results are similar to what are shown in Figure 3.4.

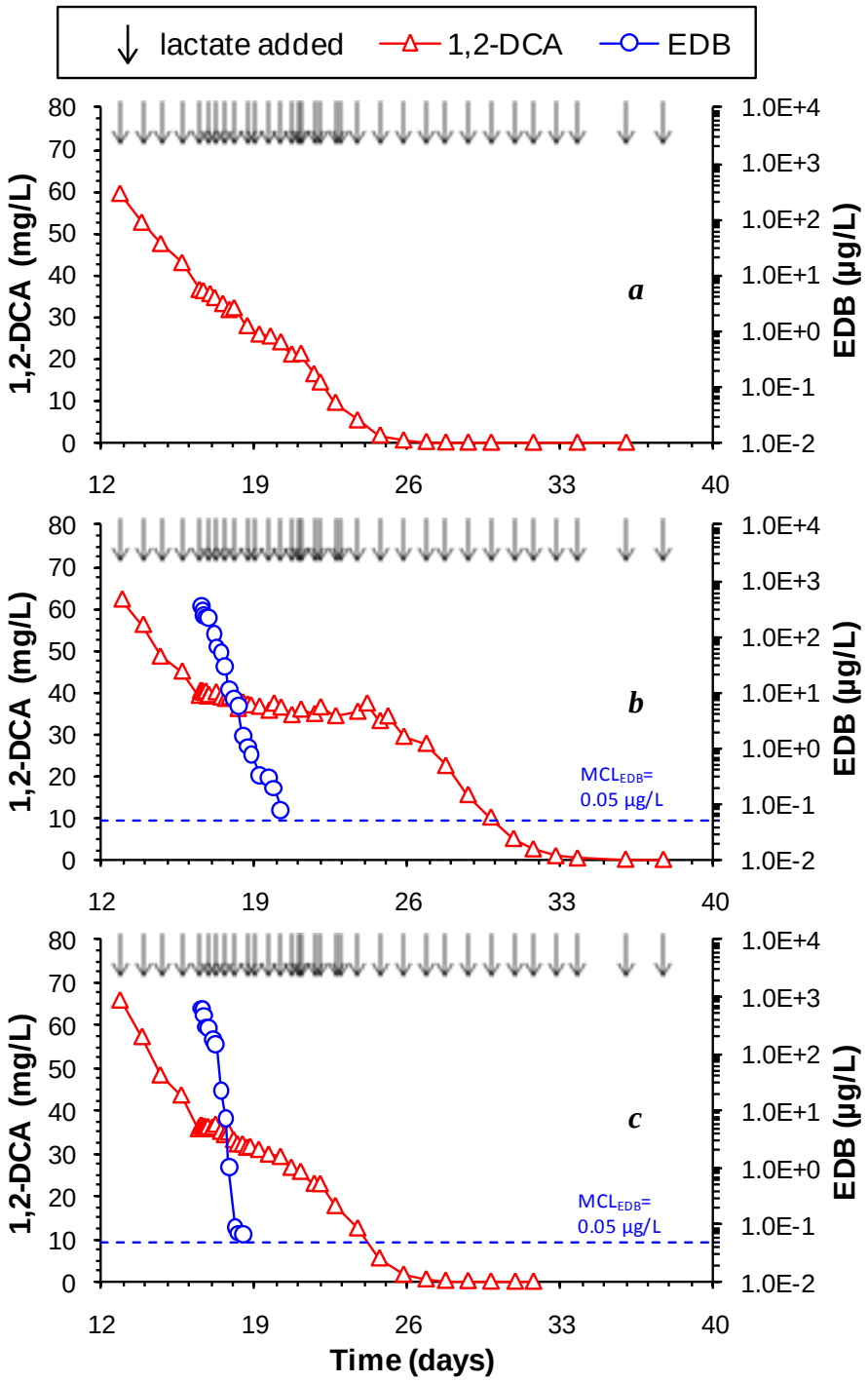


Figure A-12.1 Representative for duplicate bottles showing the effect of EDB added to the enrichment culture biodegrading high levels of 1,2-DCA with no EDB added (***a***); 370 µg/L EDB added (***b***); and 645 µg/L EDB added (***c***). Each arrow indicates addition of 0.31 mmol lactate. Replicate bottles are shown in Figure 3.4. EDB was added on day 16.5 and is shown in log scale (MCL = 0.05 µg/L).

Appendix-13: GC Response Factors

Response factors for the volatile organic compounds monitored by gas chromatography with the flame ionization detector (FID) and the electron capture detector (ECD) are provided in Table A-13.1 and A-13.2, respectively.

Table A-13.1 GC Response Factors for Volatile Organic Compounds with the FID Detector

Compound	GC RT (min)	FID Response Factor µmol/bottle /PAU[b]	R^2	Conversion Factor[a] µmol/bottle to µM	µmol/bottle to µg/L	Ref. for H_C (corresponding temperature)
Methane	0.59	2.014E-06	0.9988	0.5789	9.262	(*27*) (25 ℃)
Ethene	0.86	1.168E-06	0.9998	1.8713	52.40	(*26*) (23 ℃)
Ethane	0.95	9.700E-07	0.9998	0.8787	26.36	(*26*) (23 ℃)
VC	3.17	2.703E-06	0.9992	6.2745	392.2	(*40*) (23 ℃)
Chloroethane	3.93	4.593E-06	0.9992	7.9605	515.0	(*40*) (23 ℃)
VB	4.91	4.570E-06	0.9910	7.6824	821.6	(*28*) (25 ℃)
Bromoethane	5.55	7.064E-06	0.9999	8.4638	922.3	(*27*) (25 ℃)
1,2-DCA	8.16	4.327E-05	0.9992	9.6920	959.1	(*25*) (23 ℃)
EDB	12.46	8.610E-05	0.9999	9.8545	1843	(*25*) (23 ℃)

[a]Based on a liquid volume of 100 mL and a gas volume of 60 mLat respective temperatures.
[b] PAU = peak area unit.

Table A-13.2 GC Response Factors for Volatile Organic Compounds with the ECD Detector

Compound	GC RT (min)	ECD Response Factor µmol/bottle /PAU[b]	R^2	Conversion Factor[a] µmol/bottle to µM	µmol/bottle to µg/L	Ref. for H_C (corresponding temperature)
1,2-DCA	13.1895	7.61E-06	0.9874	9.692	959.1	(*25*) (23 ℃)
EDB	17.023	1.28E-07	0.9993	9.8545	1843	(*25*) (23 ℃)

[a]Based on a liquid volume of 100 mL and a gas volume of 60 mL at respective temperatures.
[b] PAU = peak area unit.

REFERENCES

(1) Henderson, J. K.; Freedman, D. L.; Falta, R. W.; Kuder, T.; Wilson, J. T. Anaerobic biodegradation of ethylene dibromide and 1,2-dichloroethane in the presence of fuel hydrocarbons. *Environ. Sci. Technol.* **2008,***42* (3), 864-70.

(2) ATSDR. 1995. ToxFAQs for 1,2-dibromoethane. Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/tfacts37.pdf (accessed 2010).

(3) ATSDR. 2001. ToxFAQs for 1,2-dichloroethane. Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/tfacts38.pdf (accessed 2010).

(4) U.S. EPA. 2006. Lead Scavengers Compendium: Overview of Properties, Occurrence, and Remedial Technologies. www.epa.gov/swerust1/cat/PBCOMPND.HTM (accessed 2010).

(5) U.S. EPA. 2003. Occurrence Estimation Methodology and Occurrence Findings Report for the Six-Year Review of Existing National Primary Drinking Water Regulations. EPA-815-R-03-006.www.epa.gov/safewater/standard/review/pdfs/support_6yr_occurancemethods_final.pdf. (accessed 2010).

(6) Falta, R. W.; Bulsara, N.; Henderson, J. K.; Mayer, R. A. Leaded-gasoline additives still contaminate groundwater. *Environ. Sci. Technol.* **2005,***39* (18), 378a-384a.

(7) Falta, R. W. The potential for ground water contamination by the gasoline lead scavengers ethylene dibromide and 1,2-dichloroethane. *Ground Water Monit. R.* **2004,***24* (3), 76-87.

(8) Thomason, M. M.; Vidumsky, J. E. Field evidence for natural attenuation of 1,2-dichloroethane and 1,2-dibromoethane, In *Seventh International In Situ and On-Site Bioremediation Symposium*, Magar, V. S.; Kelley, M. E., Battelle Press: Orlando, FL, June 2-5, 2003.

(9) Maymo-Gatell, X.; Anguish, T.; Zinder, S. H. Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by "*Dehalococcoides ethenogenes*" 195. *Appl. Environ. Microbiol.* **1999,***65* (7), 3108-3113.

(10) Maymo-Gatell, X.; Chien, Y. T.; Gossett, J. M.; Zinder, S. H. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* **1997,***276* (5318), 1568-1571.

(11) Kassenga, G.; Pardue, J. H.; Moe, W. M.; Bowman, K. S. Hydrogen thresholds as indicators of dehalorespiration in constructed treatment wetlands. *Environ. Sci. Technol.* **2004,***38* (4), 1024-1030.

(12) He, J. Z.; Ritalahti, K. M.; Yang, K. L.; Koenigsberg, S. S.; Loffler, F. E. Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium. *Nature* **2003,***424* (6944), 62-65.

(13) Grostern, A.; Edwards, E. A. Growth of *Dehalobacter* and *Dehalococcoides* spp. during degradation of chlorinated ethanes. *Appl. Environ. Microbiol.* **2006,***72* (1), 428-36.

(14) Duhamel, M.; Edwards, E. A. Growth and yields of dechlorinators, acetogens, and methanogens during reductive dechlorination of chlorinated ethenes and dihaloelimination of 1 ,2-dichloroethane. *Environ. Sci. Technol.* **2007,***41* (7), 2303-10.

(15) Magnuson, J. K.; Romine, M. F.; Burris, D. R.; Kingsley, M. T. Trichloroethene reductive dehalogenase from *Dehalococcoides ethnogenes*: sequence of *tceA* and substrate range characterization. *Appl. Environ. Microbiol.* **2000,***66* (12), 5141-5147.

(16) Grostern, A.; Edwards, E. A. Characterization of a *Dehalobacter* coculture that dechlorinates 1,2-dichloroethane to ethene and identification of the putative reductive dehalogenase gene. *Appl. Environ. Microbiol.* **2009,***75* (9), 2684-2693.

(17) Maes, A.; van Raemdonck, H.; Smith, K.; Ossieur, W.; Lebbe, L.; Verstraete, W. Transport and activity of *Desulfitobacterium dichloroeliminans* strain DCA1 during bioaugmentation of 1,2-DCA-contaminated groundwater. *Environ. Sci. Technol.* **2006,***40* (17), 5544-5552.

(18) Van Raemdonck, H.; Maes, A.; Ossieur, W.; Verthe, K.; Boon, N. Real time PCR quantification in groundwater of the dehalorespiring *Desulfitobacterium dichloroeliminans* strain DCA1. *J. Microbiol. Meth.* **2006,***67* (2), 294-303.

(19) Marzorati, M.; de Ferra, F.; Van Raemdonck, H.; Borin, S.; Allifranchini, E.; Carpani, G.; Serbolisca, L.; Verstraete, W.; Boon, N.; Daffonchio, D. A novel reductive dehalogenase, identified in a contaminated groundwater enrichment culture and in *Desulfitobacterium dichloroeliminans* strain DCA1 is linked to dehalogenation of 1,2-dichloroethane. *Appl. Environ. Microbiol.* **2007,***73* (9), 2990-2999.

(20) De Wildeman, S.; Linthout, G.; Van Langenhove, H.; Verstraete, W. Complete lab-scale detoxification of groundwater containing 1,2-dichloroethane. *Appl. Microbiol. Biot.* **2004,***63* (5), 609-612.

(21) De Wildeman, S.; Diekert, G.; Van Langenhove, H.; Verstraete, W. Stereoselective microbial dehalorespiration with vicinal dichlorinated alkanes. *Appl. Environ. Microbiol.* **2003,***69* (9), 5643-5647.

(22) Aronson, D.; Howard., P. H. *The Environmental Behavior of Ethylene Dibromide and 1,2-Dichloroethane in Surface Water, Soil, and Groundwater*; 4774; American Petroleum Institute, Washington, D.C.: 2008.

(23) Wilson, J. T.; Banks, K.; Earle, R. C.; He, Y.; Kuder, T.; Adair, C. *Natural Attenuation of the Lead Scavengers 1,2-Dibromoethane (EDB) and 1,2-Dichloroethane (1,2-DCA) at Motor Fuel Release Sites and Implications for Risk Management*; EPA 600/R-08/107; US EPA, Office of Research and Development, National Risk Management Research Laboratory, Ada, Oklahoma 74820: 2008.

(24) Gossett, J. M. Measurement of Henry's law constants for C1 and C2 chlorinated hydrocarbons. *Environ. Sci. Technol.* **1987,***21*, 202-208.

(25) LaGrega, M. D.; Buckingham, P. L.; Evans, J. C., *Hazardous Waste Management, 2nd edition*. McGraw Hill: New York, NY, 2001.

(26) Freedman, D. L.; Herz, S. D. Use of ethylene and ethane as primary substrates for aerobic cometabolism of vinyl chloride. *Wat. Environ. Res.* **1996,***68* (3), 320-328.

(27) SRC. 2010. Physical Properties Database Interactive PhysProp Database Demo. http://srcinc.com/what-we-do/databaseforms.aspx?id=386 (accessed 2010).

(28) Meylan, W. M.; Howard, P. H. Bond contribution method for estimating Henrys law constants. *Environ. Toxicol. Chem.* **1991,***10* (10), 1283-1293.

(29) Coleman, N. V.; Mattes, T. E.; Gossett, J. M.; Spain, J. C. Biodegradation of cis-dichloroethene as the sole carbon source by a beta-proteobacterium. *Appl. Environ. Microbiol.* **2002,***68* (6), 2726-2730.

(30) Eaddy, A. Scale-up and characterization of an enrichment culture for bioaugmentation of the p-area chlorinated ethene plume at the Savannah river site. Master Dissertation. Clemson University: Clemson, SC, 2008.

(31) Brown, S. C.; Grady, C. P. L.; Tabak, H. H. Biodegradation kinetics of substituted phenolics - demonstration of a protocol based on electrolytic respirometry. *Water Res.* **1990,***24* (7), 853-861.

(32) Verce, M. F.; Ulrich, R. L.; Freedman, D. L. Characterization of an isolate that uses vinyl chloride as a growth substrate under aerobic conditions. *Appl. Environ. Microbiol.* **2000,***66* (8), 3535-3542.

(33) Reichert, P. Aquasim - a tool for simulation and data-analysis of aquatic systems. *Water Sci. Technol.* **1994,***30* (2), 21-30.

(34) Henderson, J. K. Anaerobic biodegradation of ethylene dibromide and 1,2-dichloroethane in the presence of fuel hydrocarbons. Doctoral Dissertation. Clemson University: Clemson, SC, 2008.

(35) Bedard, D. L. A case study for microbial biodegradation: anaerobic bacterial reductive dechlorination of polychlorinated biphenyls-from sediment to eefined medium. *Annu. Rev. Microbiol.* **2008,***62*, 253-270.

(36) Yu, S. H.; Dolan, M. E.; Semprini, L. Kinetics and inhibition of reductive dechlorination of chlorinated ethylenes by two different mixed cultures. *Environ. Sci. Technol.* **2005,***39* (1), 195-205.

(37) Tandoi, V.; Distefano, T. D.; Bowser, P. A.; Gossett, J. M.; Zinder, S. H. Reductive dehalogenation of chlorinated ethenes and halogenated ethanes by a high-rate anaerobic enrichment culture. *Environ. Sci. Technol.* **1994,***28* (5), 973-979.

(38) Yu, S.; Semprini, L. Kinetics and modeling of reductive dechlorination at high PCE and TCE concentrations. *Biotechnol. Bioeng.* **2004,***88* (4), 451-464.

(39) Coleman, N. V.; Mattes, T. E.; Gossett, J. M.; Spain, J. C. Phylogenetic and kinetic diversity of aerobic vinyl chloride-assimilating bacteria from contaminated sites. *Appl. Environ. Microbiol.* **2002,***68* (12), 6162-6171.

(40) Gossett, J. M. Measurement of Henry's Law constants for C_1 and C_2 chlorinated hydrocarbons. *Environ. Sci. Technol.* **1987,***21* (12), 202-208.